OSMOREGULATORY PATTERNS AND ENERGETIC DEMANDS ON JUVENILE STRIPED MULLET (MUGIL CEPHALUS) DURING ABRUPT AND GRADUAL CHANGES IN SALINITY

BY

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Abstract of Dissertation Presented to the Graduate School
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OSMOREGULATORY PATTERNS AND ENERGETIC DEMANDS ON JUVENILE STRIPED MULLET (MUGIL CEPHALUS) DURING ABRUPT AND GRADUAL CHANGES IN SALINITY

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This study examined the effects of both gradual and abrupt short-term salinity changes on the total plasma osmotic concentrations, plasma chloride concentrations, and oxygen consumption rates of juvenile striped mullet (Mugil cephalus). The study was designed and the results were interpreted based on the predictions that emerge from a thermodynamic work model that expresses the relationship between the magnitude of the diffusion gradient and the work required to oppose this gradient in order for the organism to achieve stable regulation. When juvenile striped mullet were brought into the laboratory and held under high salinity (approximately 1000 mOsm/kg) or low salinity (approximately 100 mOsm/kg) conditions for a minimum of one week, no differences in total plasma osmolality or plasma chloride concentrations were found between the two groups. Salinity was systematically varied over a six-hour interval using a three-factor (Change x Group x Time), between-subjects design to determine whether the direction or temporal patterning of salinity change had differential effects on blood chemistry or oxygen consumption. The results indicated that abrupt or gradual decreases in the external medium's concentration over the six-hour interval had no effect. In contrast, both abrupt and gradual increases in the concentration of the medium produced increases in both total osmolality and chloride concentration.

The experimental design was extended to look at two measures (oxygen consumption and lactate production) that might indicate whether net changes in blood chemistry were also associated with changes in either aerobic or anaerobic metabolism. The results indicated that salinity decreases produced no aerobic metabolic effect; salinity increases produced increases in aerobic metabolism. None of the manipulations produced changes in the amount of lactate produced, suggesting that anaerobic mechanisms were not important in responding to these environmental changes. The results of the blood chemistry studies demonstrate that larger perturbations were observed during salinity changes which are increasing the magnitude of the osmotic and ionic gradients between these fish and their environment, than were seen during changes which were decreasing these gradients. Correspondingly, oxygen consumption rates at a given salinity can be different for animals that have experienced different salinity patterns previously.

CHAPTER 1
GENERAL INTRODUCTION

Estuarine-dependent fishes include species that inhabit estuaries for their entire lives and species that utilize these brackish water habitats only during restricted stages in their life histories. These fishes form important components of both commercial and recreational fisheries. The families Mugilidae, Sciaenidae, Clupeidae, and Engraulidae account for the dominant fisheries along much of the southeastern United States coast when measured in terms of either total biomass or numbers of individuals caught (see Cowan & Shaw, 1988).

The larvae and juveniles of many coastal marine species use estuaries as nursery habitats. The striped mullet (Mugil cephalus) is one species that conforms to this pattern of estuarine habitat usage. Several hypotheses have been proposed to explain why estuarine habitats might be attractive to young fishes. Among these are views proposing that estuaries are attractive because they are productive systems which provide better nutrition or are better places in which to escape predation relative to open-water marine habitats (Weinstein, 1979).

Estuaries might be attractive habitats due to another of feature, salinities that are intermediate between those of seawater and freshwater habitats. The body fluids of bony fishes contain dissolved substances, principally ions, in concentrations that are lower than those of seawater and higher than those of fresh water. These fishes are subject to diffusional ion fluxes and associated water fluxes in these two environments due to osmosis.

Potts (1954) presented the first explicit quantitative model that described the relationship between the magnitude of the osmotic or ionic gradients organisms face and the energetic expense required for organisms to maintain these gradients. Krogh (1939) had been among the first to demonstrate that the maintenance of these types of gradients depended on active physiological processes. In particular, Krogh had shown that freshwater fishes were able to acquire ions from the environment and keep their blood concentration above that found in the ambient fluid. Fishes have evolved a number of mechanisms to counteract ionic and osmotic stresses; among these mechanisms are ion pumps that require some fraction of the animal's total energy expenditure. Estuarine habitats, because of their intermediate salinities, may be attractive because they minimize these stresses and the energy expenditures needed to counteract them.

Potts (1954) had noted both crustacean and molluscan examples where brackish or freshwater species had lower blood osmotic concentrations relative to closely related and fully marine forms. Because the marine forms were thought to represent the ancestral condition, lower osmotic concentrations found in brackish and freshwater animals were thought to have resulted from selection. Potts formulated a model to estimate the magnitude of this selective pressure. Using an equation derived from thermodynamic theory, the model described the amount of work necessary to counteract the loss of ions, due to diffusion, that would occur if animals living in freshwater habitats retained the presumed ancestral internal concentrations (i.e., ones that are isosmotic to seawater). By comparing this projected amount of work to the amount of work required using the known internal concentrations of these brackish and freshwater animals, Potts concluded that brackish or freshwater invertebrates had gained an energetic savings by lowering their blood concentrations relative to those of their marine relatives.

Most fishes have plasma salt concentrations that are different from those of both freshwater and marine habitats, and these animals must osmo- and ionoregulate in order to maintain a relatively consistent plasma salt content, often in the face of environmental gradients that may be large or variable (Evans, 1993). Nordlie and Leffler (1975) attempted to describe the energetics of fish osmo- and ionoregulation by investigating the relationship between external medium concentrations and oxygen consumption rates of striped mullet (Mugil cephalus) across a range of salinity that extended from freshwater to greater than seawater values. These investigators used a modification to Potts' work formula given as

$$W = R \times T \times P \times | \ln C_{int} - \ln C_{ext} |$$

where W is equal to work (calories), and R is equal to the universal gas constant (1.989 calories / mole), and T is equal to the absolute temperature (°K), and $| \ln C_{int} - \ln C_{ext} |$ is equal to the absolute value of the difference in the natural log of the concentrations (moles/liter) of the internal and external environments. The term P is equal to the effective permeability of the animal's exchange surface. Nordlie and Leffler arrived at estimates of P (thought to be primarily a function of the total exchange surface area) by directly measuring the terms in the work equation, with the exception of W which was also estimated from the oxygen consumption rate.

The application of thermodynamic principles to the processes of osmo- and ionoregulation has, until rather recently, been limited to steady-state situations (i.e., ones where measurements have been made on animals that have been acclimated to test salinities for long intervals, e.g., greater than 2 weeks). There are probably many reasons why steady-state investigations have come to predominate. It is certainly likely that steady-state investigations produce results that are less variable, making their treatment effects easier to detect. It is also likely, however, that steady-state investigations have come to predominate because many investigators have been unprepared, both in terms of the level of theoretical development and the level of technological development, to deal with the issues such as non-static designs. Instead, these investigators have simply used the designs employed in previous studies.

Euryhaline fishes, including many estuarine-dependent species, represent interesting opportunities for expanding our understanding of the dynamic nature of the processes of osmo- and ionoregulation. These fishes have become adapted to habitats where changes in the external medium concentration are one of the outstanding features. Yet, field-oriented investigators who work with these animals in these environments have also tended to skirt the issue of variability. More often than not, habitat usage data are reported in terms of mean salinity, and even the estuarine zones themselves are categorized with regard to mean or, at most, range of salinity. It seems unlikely, however, that many investigators would actually subscribe to the notion that the naturally occuring salinity variations found within estuarine habitats are little more than environmental noise.

Recently, some investigators have begun to examine the responses of euryhaline fishes under non-static conditions (Davenport and Vahl, 1979; Engel et al., 1987; Moser and Gerry, 1989; von Oertzen, 1984). The findings obtained from these dynamic-condition investigations have proven difficult to interpret. There are probably several reasons why these studies have yielded results that are problematic. None of these studies contains explicit predictions regarding the relationship between the pattern of salinity manipulations used (e.g., abrupt-change versus gradual-change) and the anticipated pattern of response of the organism. More importantly, these studies have tended to use a cyclic approach to these manipulations without an appreciation of the effects multiple cycles might have (e.g., Davenport and Vahl, 1979, Moser and Gerry, 1989). In fact, very little is known regarding the rate at which fishes make salinity adaptations because most of the earlier studies were intentionally designed to measure responses after these processes were completed. Therefore, those investigators employing cyclic dynamic-case designs have no a priori knowledge that animals are entering successive cycles under similar states of adaptation.

Very little is actually known about the way that animals respond to salinity changes in the field. The possibility exists that the actual patterning of salinity changes experienced by animals, particularly motile animals, is very different from the one that might be inferred from studies of salinity fluctuations at collecting sites. As important, the actual magnitude of the gradient that exists between the animal's internal fluids and that of the surrounding fluid at any given time may be found to be very different than that expected of good iono- and osmoregulators.

Though it would have been a difficult undertaking in the absence of technology, it is now possible to extend the type of models that Potts developed to dynamic cases with the assistance of computer-based modeling. There are several advantages to be gained from this approach. The model can provide the predicted shapes of the curves that express the relationships between factors such as gradient magnitude and energy expenditure. The computer, in turn, can provide a much less labor-intensive technique, relative to hand calculations and plots, that can incorporate both the hypothetical relationships and the existing empirical data into the generation of prediction curves expressed as real-time plots. Because of its capability to do many repetitive calculations, the computer can take into account the changes in many variables over small time intervals and, thereby, track the anticipated effects of such processes as changes in the osmotic gradient. Though computer simulations can, in some instances, mimic the behavior of some systems rather precisely, their greater value may rest with their ability to assist investigators in revising hypotheses that do not accurately describe dynamic systems.

Estuarine-dependent fishes face the potential of experiencing changes in the osmotic concentration of their fluid environment. The magnitude and temporal distribution of these changes will depend on a number of factors including both the organism's behavior and chemical events that may occur with some predictable cyclicity or that may be episodic. Though in some estuarine habitats, osmotic concentrations may exceed that of seawater, most estuarine-dependent fishes probably are able to remain in fluids that have osmotic concentrations between that of fresh water and seawater. Figure 1-1 illustrates an idealized example of the sort of change these animals might encounter and depicts the salinity regime observable when the tidal amplitude and intervening freshwater inflow are sufficiently large to permit a complete turn-over from freshwater to seawater with each tide reversal. In this case, the osmotic concentration fluctuates in a sigmoidal fashion between the two extremes. The figure also depicts an animal that has an internal fluid concentration of about one-third seawater (333 mOsm/kg) and that is regulating perfectly against this environmental change. Though the details of the figure might change depending on the characteristics of the particular habitat and species under consideration, it illustrates the fundamental differences in the

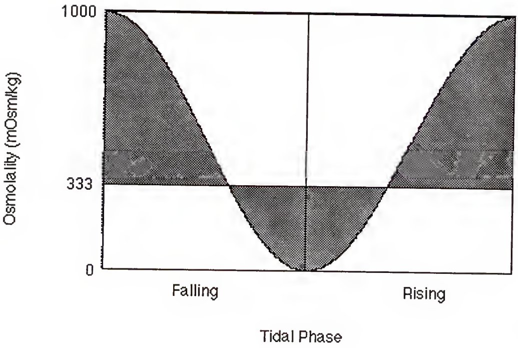

Figure 1-1. The osmotic concentration of fish blood plasma relative to the osmotic concentration of the external medium during a maximum-amplitude tidal cycle. Stable regulation of fish plasma is depicted by the horizontal line at 333 mOsm/kg. The sigmoidal tidal cycle is divided into falling and rising phases. The shaded area illustrates the difference (gradient) between the animal's internal osmotic concentration and the osmotic concentration of the external environment.

environmental challenges faced by euryhaline fishes relative to either stenohaline freshwater or marine fishes.

The task facing stenohaline fishes is, in some ways, a simpler one. These fishes must adjust their physiology in such a way that net ion and water fluxes are stabilized by balancing diffusional losses or gains through the action of opposing mechanisms. Once the actions of these mechanisms are set, they should require no further adjustment because the internal-to-external gradient remains constant. In fact, this view probably represents an over-simplification in that those same exchange surfaces involved in osmo- and ionoregulation are also involved in other processes, e.g. respiration, acid-base balance, and nitrogen excretion, that may not be proceeding at constant rates (Wood and Perry, 1985; Perry and McDonald, 1993). The dynamic influences of these different processes on one another are poorly understood.

In contrast to stenohaline forms, euryhaline fishes face the additional task of modifying their iono- and osmoregulatory mechanisms in order to match the changes in diffusional loads and losses brought about by shifts in the environmental gradient. Depending on the magnitude of the environmental change, these fishes may experience a complete reversal in the gradient, and if one considers the tidal variations and salinity stratifications that are characteristics of estuarine systems, the time-course of these adjustments may be on the order of hours or minutes. The thermodynamic model provides a starting point with which one might elucidate how these fishes are responding to these environmental events. If one assumes that these fishes are good iono- and osmoregulators, the work equation ($W = R \times T \times P \times | \ln C_{int} - \ln C_{ext} |$), can be used to generate an expected energy expenditure over time as conditions change. Using an experimental paradigm that permits good control over the other model variables, it should be possible to look at the dynamic nature of the animal's physiological adjustments even when the animal is regulating poorly and, therefore, contributing to the change in the gradient.

Experimental Paradigm and Model Predictions

Excluding the contributions of episodic events and anthropogenic influences, the osmotic and ionic characteristics of estuarine habitats are due primarily to tidal flow into and out of these systems. Sessile organisms as well as motile animals that have relatively small home ranges may experience ionic and osmotic gradients that are structured temporally by these tidal events. Motile organisms with larger home ranges may experience more rapid changes in the gradient as they move through zones of differing salinity, or they may move with tidal flow and reduce the magnitude or duration of salinity changes.

The experimental paradigm used throughout the course of this study was chosen because it permits an examination of the responses of animals that have experienced osmotic and ionic changes that are representative of those encountered in estuarine habitats. In contrast to the designs used by previous investigators, this study focused on those changes that occur as fish experience changes representing, at most, one tidal cycle. The design attempted to compare the responses of animals operating under three conditions: 1) constant salinity gradient; 2) gradual-change salinity gradient, and 3) abrupt-change salinity gradient. Figure 1-2 depicts the corresponding change in the work function, relative to the tidal regime given in Figure 1-1, that would be required of an animal attempting to regulate its internal concentration perfectly at a value of one-third seawater (333 mOsm/kg) without an alteration of the permeability term in the work equation. For comparison, the work functions for both the constant-freshwater (5 mOsm/kg) and constant-seawater

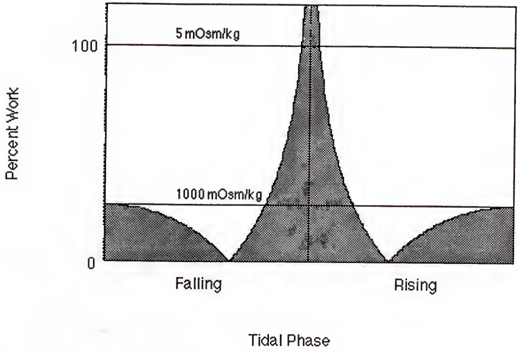

Figure 1-2. Change in work required for stable regulation of internal osmotic concentration at 333 mOsm/kg as a function of a sigmoidal change in the medium concentration between 1000 mOsm/kg and 0 mOsm/kg. Work is expressed as percent relative to the work required for stable regulation under freshwater conditions (5 mOsm/kg) as calculated from the work equation ($W = R \times T \times P \times | \ln C_{int} - \ln C_{ext} |$). The function approaches infinity as the external concentration approaches 0 at the tidal minimum between the falling and rising phases. The work function falls to 0 once during each tidal phase when the external concentration becomes isosmotic with the animal's internal concentration.

(1000 mOsm/kg) conditions over the same interval are labelled. According to the model, symmetrical tides (i.e., ones in which both the amplitude and the rate of salinity change are the same for both the rising and falling phases) should require equal amounts of work in order for the animal to regulate during each phase.

The minimum expected energetic cost for each of the three conditions employed in this study can be generated by integrating the work equation over the twelve hour interval that comprises the tidal period. Because the gradient component, $| \ln C_{int} - \ln C_{ext} |$, includes a logarithmic function, the work equation predicts that hyper-regulating against a gradient of a given magnitude and hypo-regulating against a gradient of the same magnitude will not be equally expensive. The normal gradient between the plasma of fish blood and the external medium in freshwater is presumed to be less than 400 mOsm/kg; the gradient between this plasma and the external medium in seawater is presumed to be greater than 600 mOsm/kg (Evans, 1980). Despite the fact that the freshwater gradient is about two-thirds that of the seawater gradient, the work equation predicts that hypo-regulation against the seawater gradient is nearly fifty percent less expensive relative to hyper-regulating against the freshwater gradient (see Figure 1-2). If striped mullet (Mugil cephalus) are efficient regulators against this type of gradient change, the instantaneous work function values should be accurate predictors of the change in metabolism expected under these conditions. Alternatively, if it can be shown that these animals are poor regulators under these conditions, the predicted work function can be adjusted to take into account the impact that this regulatory pattern will have on the actual magnitude of the gradient and, therefore, the required work in the absence of a corresponding adjustment in the animal's permeability.

CHAPTER 2
REVIEW OF LITERATURE

Habitat Selection by Juvenile Fishes

The factors that have been proposed as contributing to the attractiveness of estuarine habitats (i.e., coastal habitats which are intermediate in salinity between oceanic and freshwater habitats) to juvenile fishes include both physical and biological features of these environments. Many estuaries are highly productive, nutrient-rich areas where food resources are often abundant and, thereby, minimize intraspecific and interspecific competition (Weinstein, 1979). Estuaries also afford greater protection relative to open-water habitats (Boehlert and Mundy, 1988).

The decreased salinities and warmer temperatures of estuaries, at least seasonally, have also been offered as factors that explain their use as nursery areas (Hoss et al., 1988). Increased temperatures result in an increase in metabolic rate that, in turn, can translate into more rapid growth when food is not limiting. Salinities nearer to the isosmotic value reduce the osmotic and ionic gradients and, potentially, permit energy to be re-routed from osmoregulation to growth and development. In addition to its direct effects, salinity may act indirectly, through its effects on other members of the biotic community (e.g., prey species), to influence nursery habitat choices by young fishes through its effects on the food supply, substratum characteristics, and other physiochemical factors (Weinstein, 1979). Browder and Moore (1981) have proposed that the population dynamics of adult populations depend heavily on the temporal and spatial distribution of suitable juvenile habitats from one season to the next.

Boehlert and Mundy (1988) have presented a model that describes the processes that permit the successful dispersal of larval fishes into estuaries. The model seems to provide a reasonable scenario applicable to many estuarine-dependent fishes that are off-shore spawners (Cowan and Shaw, 1988; Peters and McMichael, 1987). Early in development, physical oceanographic processes (e.g., flowing currents) keep or move fish into near-shore areas. With successive morphological and physiological development, animals orient to and follow environmental stimuli that permit settlement into nursery habitats. Some species seem to use photoperiod and tidal cues to position themselves in the water column and take advantage of stratified water currents within estuaries (Weinstein et al., 1980).

Many estuarine fishes also show selective movements to different regions within estuaries as development progresses. For example, young spotted seatrout (Cynoscion nebulosus) and red drum (Sciaenops ocellatus) show high dietary overlap and are found in similar estuarine habitats. As they develop, their dietary habits diverge and they disperse to different regions within estuaries (Peters and McMichael, 1987; McMichael and Peters, 1989).

As adults, many estuarine-dependent fishes show an even greater degree of habitat specialization. For instance, fishes in the family Sciaenidae are characterized as coastal fishes, yet these fishes show a diversity of habitat selection. Along the southeastern U.S. coast, banded drum (Larimus fasciatus) are found in near-shore waters but are seldom found in estuaries (Ross, 1984). Other adult sciaenids, e.g., spot (Leiostomus xanthurus) and Atlantic croaker (Micropogonius undulatus) show stronger associations with estuarine habitats (Moser and Gerry, 1989). Red drum (Sciaenops ocellatus) and weakfish

(Cynoscion regalis) are found in near-shore and estuarine habitats (Beckman et al., 1989; Shepard and Grimes, 1983). Despite these differences in adult distribution, most sciaenids seem to be dependent on estuaries to complete some stages of early development.

Osmoregulatory Abilities and Mechanisms in Developing Fishes

The eggs of some demersal and pelagic spawning teleosts are isosmotic with maternal fluids prior to spawning (Kjorsvik et al., 1984). Teleost eggs are tolerant of a large range of salinities. Holliday and Blaxter (1960) showed that eggs of the herring (Clupea harrengus) could be fertilized successfully at salinities between approximately 6 and 53 parts per thousand (ppt). Rockwell (1956) found the optimal hatching salinity for two species of Pacific salmonids (Oncorhynchus gorbuscha and O. keta) to extend from 6 to 24 ppt. Though normally hatched in freshwater, young coho salmon (Oncorhynchus kisutch) tolerate full seawater soon after yolk sac reabsorption and months in advance of their normal time of migration to oceanic waters (Conte et al., 1966). Although tolerant of salinity variations, spotted seatrout eggs show reduced hatching success percentages as salinities are increased from 20 to 60 ppt. (Gray et al., 1991).

Upon spawning, marine teleost eggs are exposed to a greater than two-fold increase in salinity (from approximately 13 to greater than 32 ppt) as these eggs pass from the environment provided by the maternal fluids to that of the external environment. Striped mullet (Mugil cephalus) spawn in sea water (Sylvester et al., 1975). Egg survival occurs only in water with salinity near full-strength sea water (36 ppt), and newly hatched fish tolerate water that is above 24 ppt. Kurata (1959) and Holliday (1965) found that the embryos of some marine flounder and herring have internal osmotic concentrations of 22.5 and 30 ppt, respectively, soon after contacting seawater. Within 24 hours, these embryos begin to regulate their osmolalities at about 15 ppt, values that are much nearer to the range in which adults regulate. Kjorsvik et al. (1984) indicate that, in general, eggs of pelagic spawners maintain osmolalities near maternal values whereas eggs of demersal spawners show rapid rises in osmolality. Holliday (1971) proposed that the less specialized tissues of young embryos are less susceptible to the perturbing effects of ionic or osmotic swings. The mechanisms affording the reduced risk of perturbation were not identified.

Shelbourne (1957) first proposed that the osmoregulatory mechanisms utilized by early life-history stages of marine fishes likely would be different from those utilized by adults. He anticipated that larval forms would show a greater dependence on surfaces other than their gills and suggested that the sites of ion exchange would involve integumentary or embryonic membranes. Hwang and Hirano (1985) have demonstrated the yolk sac epithelium to be functioning in this respect. This membrane possesses chloride cells and other structural components typical of ion exchange tissues. Kjorsvik et al. (1984) have shown that eggs that are capable of holding osmolalities near maternal values following spawning possess vitelline membranes that are impermeable to both ions and water.

Whitfield (1990) surveyed the species of fishes found in some South African estuaries and categorized them as either full-time estuarine residents or estuarine transients, the latter including species using estuaries as nursery areas. In this scheme, estuarine residents are typified by spawning relatively few, demersal eggs and they may engage in parental care. Estuarine transients that reproduce in oceanic waters spawn small, pelagic eggs and show no parental care. Though predicted to produce more altricial young (e.g., animals with absent or poorly developed feeding and movement-related structures and relatively large yolk sacs) based on the premise that estuaries are more physically unstable environments relative to oceanic habitats, estuarine residents were found, in many cases, to produce relatively precocial hatchlings.

Oikawa et al. (1991) also have characterized hatchlings of demersal spawners as being relatively precocial. These investigators provide data that indicate that caution should be exercised in comparing metabolic data that are not obtained from embryos or larvae at similar developmental stages. Sea bream larvae (Parus major) older than 27 days show mass specific metabolic rates that decrease with increasing mass as seen in most other organisms. Larvae between 8 and 27 days post-hatching show constant rates of mass specific metabolism with increasing mass, while larvae less than 8 days post-hatching show increases in mass specific metabolic rates with increasing mass. These researchers attribute these differences to the development of different tissue types in larvae of different ages. Tissues with high metabolic demands (e.g., neural tissue) account for much of the development seen in young larvae whereas tissues with lower metabolic demands (e.g., white muscle and bone) are developing in older larvae.

Juvenile fishes (i.e., ones that are post-metamorphic and possess morphology similar to that of older animals) probably depend on the same mechanisms that adult fishes utilize to iono- and osmoregulate. As such, cortisol and prolactin likely integrate the long-term adjustments in physiology that these fishes make when living in more saline or freshwater environments, respectively (see review by Foskett et al., 1983). Some juvenile fishes (i.e., juvenile salmonids that are diadromous) employ endocrine regulators that may not function in osmoregulation in other species. Although cortisol seems to be responsible for the differentiation and proliferation of chloride cells in the gills of salmonid juveniles that are migrating seaward, thyroid hormones and growth hormone seem to be necessary for full functioning of gill tissue as an osmoregulatory apparatus (e.g., production of adequate levels of gill sodium-potasium- ATPases) (Richman et al., 1987; Richman and Zaugg, 1987). The adaptations that are expressed as juvenile salmonids prepare to migrate seaward enable these fishes to tolerate the higher salinity of the open ocean until they make the return migration to their natal stream, an interval that may last several years. In contrast, many other estuarine-dependent fishes must make physiological adjustments that may only be required for hours or days. The chemical regulation of these shorter-term adjustments is largely undescribed.

Effects of Varying Salinity on Ionic and Osmotic Balance

The time-course for osmotic adjustment to salinity changes extends from minutes for some fishes to hours or days for others (Holliday, 1971). Maximal ionic and osmotic disturbances would be expected to result from abrupt transfer between different salinities. Bath and Eddy (1979) transferred adult rainbow trout (Oncorhynchus mykiss, formerly Salmo gairdneri) weighing approximately 350 grams from freshwater to seawater. Plasma sodium levels did not change, but chloride levels were elevated by 5 hours after transfer and remained up for 24 hours. During this period, oxygen consumption declined to 75 percent of freshwater values. The reduction in oxygen consumption was hypothesized to result from the dehydrating effect (i.e., the loss of water from tissue in response to the osmotic gradient) of the medium on gill tissue, perhaps causing lamellar membranes to collapse. Leray et al. (1981) found both plasma sodium and chloride to be elevated two to four hours following transfer of rainbow trout to seawater. These ions remained elevated for 30 hours, and up to six days were required for complete adjustment to baseline.

Engel et al. (1987) transferred Atlantic menhaden (Brevoortia tyranus) in increasing, 3.5 to 35.0 ppt, and decreasing, 35.0 to 3.5 ppt, directions. Plasma sodium, chloride, and osmolality decreased within 30 minutes following transfer to the lower salinity and remained low for 24 hours. The reverse effect was seen with transfer to the higher salinity though the magnitude of the changes was not as large as that seen during salinity decreases. Complete ionic and osmotic adjustment was achieved at 120 hours.

Milkfish (Chanos chanos) raised at 32 ppt and transferred to four salinities between 0 and 48 ppt also exhibited changes in both plasma chloride and osmolality (Ferraris et al., 1988) with smaller fish showing greater changes than larger fish. Leray et al. (1981) have proposed that laboratory-induced abrupt transfers, which may be representative of salinity changes encountered in the field by motile animals, result in a crisis period, lasting for approximately 30 hours, during which the animal must mobilize the mechanisms to respond to the change in the environment, followed by a 6 to 10 day period for full osmotic adjustment. Under natural conditions, many euryhaline fishes face not only changes in the mean salinity, but they also face changes in the variance of salinity around the mean (Williams and Williams, 1991).

In addition to perturbing blood ion concentrations, abrupt exposure to water of increased salinity has been hypothesized or shown to have other effects, both direct and indirect, on physiology. Increasing the salinity of water both changes its specific gravity (i.e., its density) and lowers its capacity to hold dissolved oxygen. Holliday (1971) proposed that increases in specific gravity would translate into increases in the cost of locomotion and activity and that decreases in the oxygen content could lead to asphyxiation. Rao (1971) found a decreased oxygen consumption by rainbow trout (Oncorhynchus mykiss) proportional to the increased salinity to which they were exposed. This pattern is contrary to that predicted by the thermodynamic model of Potts (1954) and was hypothesized to result from a reduction in gill transfer factor (surface area times diffusion distance) with gill dehydration. Bath and Eddy (1979) noted that the behavior of rainbow trout abruptly transferred to seawater indicated potential respiratory distress resulting from gill dehydration. Morgan and Iwama (1991) have concluded that any direct effects of salinity on metabolism are small when compared to secondary effects working through endocrine and other physiological processes; these evolutionary adaptations can alter the expected effect of salinity on metabolism.

The literature describing the control of osmoregulatory mechanisms is extensive; reviews of this literature are to be found in papers by Foskett et al. (1983), and Pang (1983). Evans (1984) points out that the mechanisms different fishes use for ion and osmotic regulation are similar. The functioning of branchial, renal, digestive, and integumentary components to achieve regulation by fishes in hyposaline versus hypersaline environments is reviewed by Evans (1980, 1993). More detailed review of the cellular level functioning of these mechanisms is given in Evans (1984). Juvenile fishes (i. e., ones that are post-metamorphic) are presumed to rely on the same osmoregulatory mechanisms utilized by adults, but their smaller size potentially subjects them to larger salinity effects.

Huntsman and Hoar (1939) were the first to demonstrate that the ability of Atlantic salmon to adapt to seawater was related to size; smaller animals were unable to survive in this environment. Hickman (1959) also showed that the osmoregulatory ability of the starry flounder (Platichthys stellatus) was related to size. Eddy and Bath (1979) demonstrated that smaller rainbow trout (Oncorhynchus mykiss) were more affected by transfer from freshwater to seawater, as evidenced by greater changes in blood ion concentration and osmolality, and that the smallest individuals were unable to fully adapt to full-strength seawater. Heifetz et al. (1989) found that sockeye salmon (Oncorhynchus nerka) less than 50 mm are also unable to tolerate seawater. Bath and Eddy (1979) attributed this greater susceptibility of smaller fish to ionic and osmotic disturbance and their poorer ability to regulate against a seawater challenge to their greater surface area to volume ratios.

The potential role of size in the osmoregulatory process has also been suggested based on the results of studies conducted on animals that normally inhabit low salinity environments. Using the rate of depletion of stored fat as an index of energetic cost, Toneys and Coble (1980) found that smaller yellow perch, a freshwater species, expended more energy than larger ones as salinity was elevated above fresh water. Beamish (1980) compared lampreys restricted to bodies of fresh water with those that normally encounter higher salinities. Land-locked animals were smaller in size and were unable to tolerate exposure to seawater. The author proposed small size as a potential factor in these fish's greater susceptibility to seawater disturbance.

Nordlie et al. (1982) examined the effect of salinity on the osmoregulatory abilities of juvenile striped mullet (Mugil cephalus) in three size classes across a range of salinity that extended from fresh water to seawater. Of these classes, animals that were between 20 and 29 mm total length (TL) were unable to tolerate transfer to fresh water. Animals in the two larger classes, 30 to 39 and 40 to 69 mm (TL), were capable of regulating across the full range. McEnroe and Cech (1985) found a direct correlation between the salinity levels that white sturgeon (Acipencer transmontanus) could adapt to and their mass.

Animals less then 1.8 grams were unable to survive salinities greater than 10 ppt. Animals up to 10 grams could adapt to 15 ppt; animals approaching 56 grams could tolerate salinities up to 25 ppt.

Energy Requirements for Osmoregulation

In 1954, Potts presented a quantitative model and some illustrative examples dealing with the relationship between osmoregulation and energetics based on thermodynamic equations. The model illustrated that small changes in internal osmolality have large effects on energetic savings when the original discrepancy between internal and external osmolalities is large. As internal osmolality approaches external osmolality, a larger internal change is required for even a small energetic savings. Eddy (1982) made thermodynamic calculations of the minimal metabolic cost of osmoregulation for fishes similar to those made earlier by Potts for aquatic invertebrates. Estimates of the percentage of total metabolism required for this process varied from 0.5% for marine forms to 1.0% for freshwater forms. Both investigators hypothesize that osmoregulatory cost should be minimal in isosmotic environments.

Estimates of the metabolic cost of osmoregulation vary considerably between studies due, in part, to differences in the measurement conditions. Von Oertzen (1984) advocates measuring osmoregulatory cost as a fraction of the standard (i.e. resting and unfed) metabolic rate as opposed to either the routine or active metabolic rate. Other investigators advocate assessing the cost via its effect on metabolic scope (i.e. the difference between resting and active metabolic rate) (Vahl & Davenport, 1979; Febry & Lutz, 1987; Perez-Pinzon & Lutz, 1991).

Farmer and Beamish (1969) estimated that 30% of total metabolic energy is devoted to osmoregulation in the Nile tilapia (*Tilapia nilotica*) with changing salinity, and that 20% is consumed under isosmotic conditions. Woo and Tong (1982) found correlated elevations of oxygen consumption and gill ATPase activity of the snakehead (*Ophiocephalus maculatus*) with exposure to increasing salinity. Looking at three species of Australian fishes that normally inhabit estuarine environments, Langdon (1987) found increases in both ATPase and mitochondrial succinate dehydrogenase activities when these species were exposed to seawater. Gill ATPase activity was lowest for *Cyprinodon salinus*, a North American cyprinodontid, at a salinity of one half seawater, the value nearest to the isosmotic point used in their study. ATPase activity was elevated at freshwater, seawater, and one and one half times seawater. Branchial energetic demand to replace lost ions in stenohaline catfishes (*Ictalurus nebulosus* and *I. punctatus*) was estimated at 5% of osmoregulatory cost with most of the remaining cost attributable to renal ion conservation (Furspan et al., 1984).

Resting or routine metabolism measurements have not consistently yielded results indicating that osmoregulatory cost is lowest near the isosmotic point. Moser and Gerry (1989) were unable to show a correlation between oxygen consumption and salinity for either the spot (*Leiostomus xanthurus*) or croaker (*Micropogon undulatus*) despite field data indicating that both species have habitat preferences that are related to salinity. Moser and Hettler (1989) examined the relationships between a number of factors that were expected to influence the metabolism of young spot (*Leiostomus xanthurus*). Both temperature and salinity were found to be directly related to the metabolic cost of osmoregulation with the per-degree thermal increase about five times greater than the part-per-thousand salinity increase.

Two other sciaenids, however, do show oxygen consumption patterns similar to that predicted by the thermodynamic model. Both spotted seatrout (Cynoscion nebulosus) and red drum (Sciaenops ocellatus) do show oxygen consumption minima at salinities intermediate between freshwater and seawater with increasing rates as salinities approach either extreme (Wohlschlag & Wakeman, 1978; Wakeman & Wohlschlag, 1983). Nordlie and Leffler (1975) exposed mullet (Mugil cephalus) to a range of salinities from freshwater (5 mOsm/kg) to greater than seawater. Oxygen consumption was not different in the range of salinities from freshwater (5 mOsm/kg) to twice the isosmotic value (25 mOsm/kg), but oxygen consumption did increase at salinities above this range. Nordlie (1978) has proposed classifying fishes into four categories according to their metabolic responses to different salinities. Oxygen consumption patterns for many species are summarized by Nordlie (1978) and by Morgan and Iwama (1991).

Vahl and Davenport (1979) first suggested that the cost of osmoregulation may only be apparent when measured as a fraction of the active metabolic rate. Blennies (Blennius pholis) defend territories and face osmoregulation stress during tide-in periods that result from adjusting their respiratory physiology to meet gas exchange requirements. During low tide, these animals are able to buffer salinity changes by burrowing into the sediment and remaining inactive.

Wood and Perry (1985) review the effects of activity on metabolic rate and the physiological adjustments necessary to support this activity. Febry and Lutz (1987) forced tilapia to swim at different speeds and compared oxygen consumption as a function of varying salinity at each speed. At lower speeds, no relationship between oxygen consumption and salinity was found. With increasing speed, osmoregulatory cost became apparent; oxygen consumption was minimal near the isosmotic value and increased as salinity either decreased or increased away from this point. Perez-Pinzon and Lutz (1991) have shown a similar response for juvenile snook (Centropomus undecimalis) and have proposed that the energetic requirements for osmoregulation put these fish at risk when foraging or escaping predators at higher salinities.

Another index of the cost of osmoregulation is the effect of salinity on growth. De Silva et al. (1986) found that optimal growth of larval Oreochromis niloticus occurred at a salinity of 11.4 ppt, a value very near to the isosmotic point. Both coho salmon (Oncorhynchus kisutch) and Baltic salmon (Salmo salar) show reduced growth and impairment of osmoregulatory ability with early exposure to seawater (Bolton et al., 1987; Virtanen et al., 1988). Morgan and Iwama (1991) have shown that exposure to salinities above the isosmotic point have a negative effect on growth rates of Oncorhynchus mykiss and Oncorhynchus tschawytscha. Recently, Konstantinov and Martynova (1990) have shown that the interaction of salinity with growth is more complex than predicted by thermodynamic theory. Constant salinities that yielded optimal growth were first identified for five fish species including a sturgeon and four teleosts. Subsequent procedures involving temporal variations of salinity demonstrated that all of these species grew better under variable rather than constant salinity regimes. The magnitude of the optimal variation was directly related to the degree of euryhalinity of each species, but these investigators offered no hypothesis regarding the mechanism producing the effect.

Methodological Considerations: Respirometry of Aquatic Organisms

Manometric or volumetric, closed-system, and open-system techniques have all been used to to measure the oxygen consumption rates of aquatic organisms under various conditions. Each technique has its own set of advantages and disadvantages that should be assessed in selecting the one appropriate to a particular experimental paradigm. These features are addressed by Laughlin et al. (1979) and by Steffensen (1989). Manometric or volumetric techniques have not been widely used probably owing to the excessive shaking usually required to achieve air-water equilibrium that can disturb animals and result in highly variable results (Laughlin et al., 1979).

Closed-system measurements have been widely used to study fish respiration. The technique is sensitive, providing an appropriate animal size to chamber volume ratio is chosen. Accuracy declines with small changes in water content brought about by a low ratio. Closed systems are subject to water stratification of gas content if not mixed adequately, and this stratification can produce measurement error. Accumulations of carbon dioxide and other metabolites and decreasing oxygen partial pressures all can produce changes in respiration rates of aquatic organisms (Steffensen, 1989).

Ege and Krogh (1914) were the first to apply the open-system respirometry technique to fish. In their study, flow rates were adjusted such that animals extracted about 20 percent of the oxygen from the water flowing through the chamber. This percentage can be adjusted in open systems by changing either the flow rate or chamber volume. A problem that has led to the repeated incorrect interpretation of open-system respirometry data is the failure to take into account the wash-out or reservoir lag inherent in this type of system. Fry (1971) provides a method of correcting wash-out lags brought about by the system's dilution factor (i.e. the ratio of the flow rate to the chamber volume). More recently, Niimi (1978) has provided a mathematical formula for approximating oxygen consumption rate when the system is not at steady state.

Several factors produce variability in oxygen consumption rate measurements whether measured in closed or open systems. These factors should be controlled to maximize accurate detection of rate differences with changes in environmental conditions (e.g., salinity). Both oxygen electrodes and microbes can consume oxygen, and control runs made with the experimental animal absent from the chamber should be performed to allow correction for the other effects (Laughlin et al., 1979). Filtering water through millipore filters prior to its use can reduce microbial consumption.

Respiratory demands associated with the digestion and assimilation of food can also bias results. Vahl and Davenport (1979) found this demand, specific dynamic action, to peak between two and four hours post-feeding in Blennius pholis, but oxygen consumption did not completely return to baseline for several days. Most metabolic studies of fishes involve measurement of standard metabolic rate (i. e., measurements made on resting and unfed animals), but the period that these animals have been denied access to food has varied considerably between studies. Animals routinely are not fed for 24 hours prior to measurement, but intervals as short as 8 hours and as long as 2 weeks have been used (Moser & Gerry, 1989; Vahl & Davenport, 1979).

Spontaneous activity by animals in chambers can greatly confound experimental results. Most studies attempt to minimize this effect by allowing the animal to adapt to the apparatus for some time interval prior to measurement. In some studies, the apparatus is constructed so as to minimize animal movement (e.g. Wakeman & Wohlschlag, 1983). Though dark chambers are often used to reduce activity during the normal light-on period, Steffensen (1989) recommends making measurements during the animal's normal dark period of the animal's lighting schedule when, in diurnal animals, their spontaneous activity tends to be lower and less variable.

Blazka et al. (1960) introduced a technique that forces fish to swim in stationary chambers by varying the flow of water through the chamber. This technique permits the assessment of the metabolic cost associated with locomotion. Morgan and Iwama (1991) have used a modification of this technique to standardize the effects of fish activity when attempting to investigate salinity effects. In their study, fish were forced to swim at one body length per second in order to minimize random activity shown by fish while ostensibly at rest. Febry and Lutz (1987) and Perez-Pinzon and Lutz (1991) have used such chambers to vary swimming speed and to demonstrate salinity effects that were previously not apparent in data obtained from resting fishes.

CHAPTER 3
EFFECTS OF ABRUPT AND GRADUAL SALINITY CHANGES ON PLASMA OSMOTIC AND CHLORIDE CONCENTRATIONS OF JUVENILE STRIPED MULLET

Both the diffusion and work equations (presented in Chapter 1) include terms for the concentration gradient that exists between an aquatic organism's internal body fluid and the external medium. The magnitude of this gradient may be altered when the osmotic concentration of either the environment or the animal's internal body fluid changes. Very little is known, however, about the role of changing internal body fluid concentration in altering the magnitude of the concentration gradient as fishes are subjected to short-term (e.g., tidal) changes in the external medium's concentration. This chapter presents an empirical study to determine the effects of short-term environmental changes on the internal body fluid concentration of juvenile striped mullet (Mugil cephalus).

Methods

This study was conducted over a two-year period beginning in July 1991 and concluding in November 1993. Over four hundred juvenile striped mullet, Mugil cephalus, were caught using either a 10 m beach seine or a 6 ft cast net from locations near the Whitney Laboratory, St. Augustine, Florida. These animals were caught mainly in the intercoastal waterway that runs adjacent to the Whitney Laboratory or a few kilometers north of the laboratory in Matanzas Inlet, a channel that connects the intercoastal waterway with the Atlantic Ocean.

Fish ranged in size from 8.9 cm to 22.0 cm (TL) and were caught mainly in the months August, September, and October, though some animals were caught in July and November of each year. During August and September 1991, blood samples were taken from approximately 150 fish at the field locations where they were caught by removing the scales overlying the pectoral portion of their ventral surface, wiping the underlying skin dry, and inserting a sharpened heparinized (ammonium heparin) capillary tube immediately posterior to the pectoral girdle and through the wall of the body cavity, pericardial chamber, and heart or major vessels leading to or away from it. In addition, water samples (approximately 5 ml) from each capture site were collected in glass vials. Blood and water samples were stored on ice for return to the laboratory. Blood samples were centrifuged to remove formed elements and all samples were stored in sealed tubes at -20 °C for subsequent chemical analyses.

Beginning in September 1991 and continuing through November 1993, experiments were conducted in which environmental conditions were manipulated according to the general design presented in Table 3-1. Each collection sample of animals was returned to the laboratory and divided in half by matching animals according to their total length (TL). The ground-water salinity in the vicinity of the Whitney Laboratory necessitated using 100 mOsm/kg rather than 5 mOsm/kg water as the low-salinity water in all subsequent experiments. One half of the sample was assigned to a high (approximately 1000 mOsm) environmental condition and the other half of the sample was assigned to a low (approximately 100 mOsm) environmental condition. Animals were held in these conditions in well-aerated water for, at least, a one week period before subsequent experimentation was conducted. All experiments were conducted at approximately 25 °C between 1000 h and

Table 3-1. Three-factor between-subjects design used to determine the patterns of total plasma osmolality changes and plasma chloride concentration changes in juvenile striped mullet (Mugil cephalus) subjected to either abrupt or gradual changes in salinity Each cell gives the number of fish for a given change x group x time condition.

	Decrease		
	Abrupt	Control	Gradual
Time (hrs.)			
1	10	10	10
2	10	9	9
3	10	10	11
4	7	8	7
5	6	6	6
6	8	7	9

	Increase		
Time (hrs.)	Abrupt	Control	Gradual
1	13	9	9
2	10	10	10
3	8	10	15
4	6	6	6
5	6	6	6
6	6	6	6

1900 h during the light phase a 12:12, light:dark cycle that began at approximately 0700 h.

During each experimental session, a sub-sample of animals was removed from each of the high and low holding samples. Each of these sub-samples was further divided into three groups (abrupt-change, gradual-change, and control) by matching animals according to their total lengths. Each group was transferred to a well-aerated opaque-white plastic container (approximately 20 L) at the start of the experiment. Control animals were placed into a container with water that had the same osmotic properties as the water in which they were held during the preceding holding period, and this water remained the same for the next six hours. Abrupt-change animals were placed into a container with water of the other osmotic extreme (low-to-high or high-to-low transfer) at the start of the experiment, and this water remained the same for the next six hours. Gradual-change animals were placed into a container that had the same osmotic properties as the holding condition, but this water was replaced at a constant rate over the next six hours so that, by interval's end, these animals were immersed in water of the other osmotic extreme.

For all groups, the six-hour experimental interval was subdivided into seven sampling periods . During each sampling period, blood samples were collected from fish using a method similar to the one employed in the field. The skin overlying the pectoral region, pericardial chamber, and heart or associated vessels was pierced using a fine-pointed surgical scissor blade, and two to three capillary tubes were filled. Baseline samples were made at the start of the experiment. The remaining six sampling periods each extended from one-half hour before to one half-hour following each full-hour time-point from the start of the experiment, providing a half-hour interval between each sampling period. Within each sampling period, samples were always obtained from the same number of animals in each of the three groups (abrupt-change, gradual-change, and control) with sampling order randomized for the three groups. A total of 36 animals, 18 from the high holding group and 18 from the low holding group, was used to collect baseline samples over the course of the entire study; these samples were used to measure baseline differences between the two groups (high and low) prior to the experimental manipulations. An additional 301 animals were used to collect the one through six hour samples.

All plasma samples were prepared by centrifugation of whole blood at 10,000 rpm for 10 minutes. Plasma samples were separated from formed elements and transferred to 5 uL micropipettes (Fisher Scientific, Pittsburg, Pa.). Plasma total osmolality, chloride and sodium concentration analyses were all conducted on 5 uL replicates from each animal. Plasma total osmolality was determined using a vapor pressure osmometer (model 5100B, Wescor Inc., Logan, Ut.). Plasma chloride concentration was determined using a chloride titrator (model CMT10, Radiometer Instruments, Copenhagen). Plasma sodium concentration was determined using an atomic absorption spectrophotometer (model 2100, Perkin Elmer Inc., Norwalk, Ct.).

Results

When the blood samples collected from fish in the field were analyzed, two patterns resulted. Figure 3-1 shows a regression analysis relating the osmolality of plasma samples from these fish to the environmental osmolality of the capture sites. The slope of the regression line is not different from zero (R^2=0.005, p=0.4021). Figure 3-2 illustrates the results of a regression analysis comparing total length with plasma osmolality; this relationship suggests that

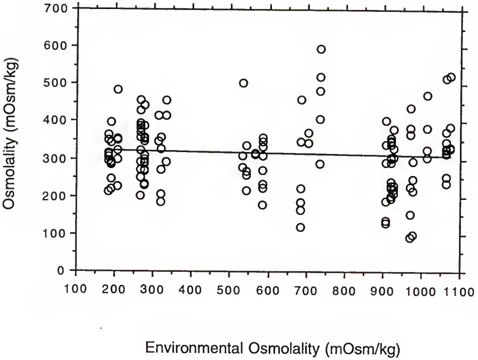

Figure 3-1. Plasma osmolality as a function of environmental osmolality for juvenile striped mullet (Mugil cephalus).

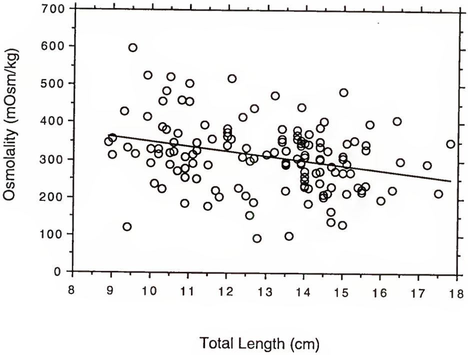

Figure 3-2. Plasma osmolality as a function of length in juvenile striped mullet (Mugil cephalus).

small animals have significantly higher plasma osmolalities relative to those of larger animals as described by the equation

$$\text{osmolality} = -12 \times \text{length} + 477$$

($R^2=0.094$, $p=0.0001$). In all subsequent experiments, fish were matched according to their total lengths and then assigned to treatment groups in a way that would preclude size from systematically interacting with the experimental variables.

Regression analysis of the relationship between plasma total osmolality and chloride concentration showed a significant positive correlation between these measures described by the line

$$\text{chloride concentration} = 0.54 \times \text{total osmolality} + 4.46$$

($R^2=1.000$, $p=0.0001$). The plasma sodium measurements were found to be unreliable, resulting from inconsistent sample volumes being aspirated into the atomic absorption spectrophotometer. This error seems to have been introduced by unidentified material in the diluted plasma samples blocking the machine's aspirator tube. Insufficient plasma volumes were obtained from each animal to permit a second run of plasma sodium measurements, so plasma sodium data have been omitted from the subsequent analyses. Table 3-2 presents the results of the three-factor (Change x Group x Time) ANOVA comparing the osmolalities of fish under each treatment condition. The results of the same ANOVA procedure applied to the plasma chloride values of these animals are given in Table 3-3. Although no significant three-way interaction

Table 3-2. Results of three-way ANOVA, Change x Group x Time, for total plasma osmolality of juvenile striped mullet (Mugil cephalus).

Source	df	F	P
Change	1	0.250	NS
Group	2	4.905	0.0081
Time	5	5.735	0.0001
Change x Group	2	4.280	0.0148
Change x Time	5	4.891	0.0003
Group x Time	10	2.376	0.0104
Change x Group x Time	10	1.294	NS
Error	265		

Table 3-3. Results of three-way ANOVA, Change x Group x Time, for total plasma chloride concentration of juvenile striped mullet (Mugil cephalus).

Source	df	F	P
Change	1	3.497	NS
Group	2	1.407	NS
Time	5	12.464	0.0001
Change x Group	2	4.627	0.0106
Change x Time	5	3.164	0.0086
Group x Time	10	2.708	0.0035
Change x Group x Time	10	1.273	NS
Error	265		

between these variables was found when either plasma osmolality or chloride was used as the dependent variable, both of these analyses reveal significant two-way interactions between all combinations of the three factors. A three-factor ANOVA using total length as the dependent variable revealed no significant differences between any of the animals in the various treatment combinations.

The plasma osmolalities and chloride concentrations of fish in the different group-time combinations under the decreasing-salinity condition are presented in Figures 3-3 and 3-4. Post-hoc analysis using the Scheffe test revealed significant differences between some of the control groups at the different time intervals for both total osmolality and chloride concentration. Figures 3-5 and 3-6 present the plasma osmolality and chloride concentrations of each of the experimental groups at each time interval referenced to their corresponding control group values. Post-hoc comparisons revealed a significant effect for time when the gradual-change and abrupt-change groups were collapsed within the different one-hour intervals. No significant group x time interaction or main effect for group was found when the two experimental groups (abrupt-change and gradual-change) were compared.

Figures 3-7 and 3-8 present the plasma osmolality and chloride concentration data for the increasing-salinity condition. No significant differences in plasma osmolality or chloride concentration were found between any of the control groups at each time interval. However, post-hoc analyses did reveal a significant group x time interaction for both plasma osmolality and chloride concentration measures indicating that the time-courses of the gradual-change and abrupt-change effects were different. Figures 3-9 and 3-10 present the osmolality and chloride concentration data for the two experimental groups referenced to their respective control groups at each time interval.

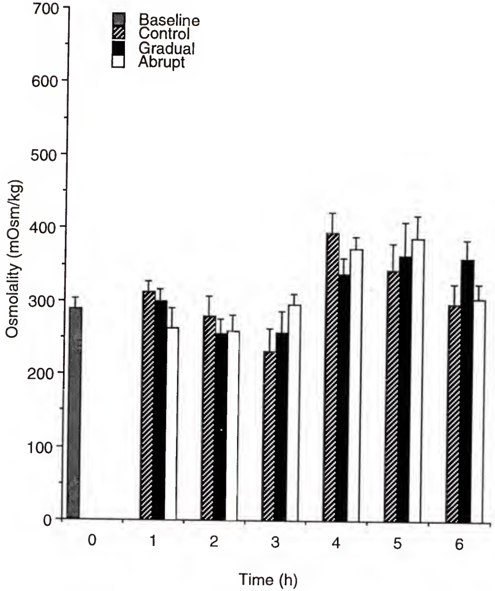

Figure 3-3. Plasma osmolality over six hours during salinity decreases.

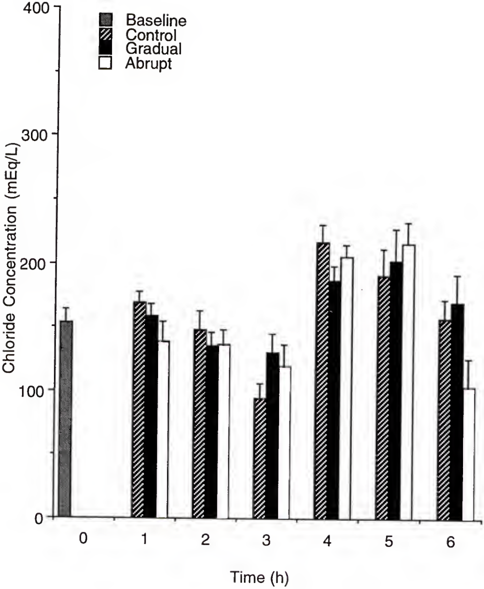

Figure 3-4. Plasma chloride concentration over six hours during salinity decreases.

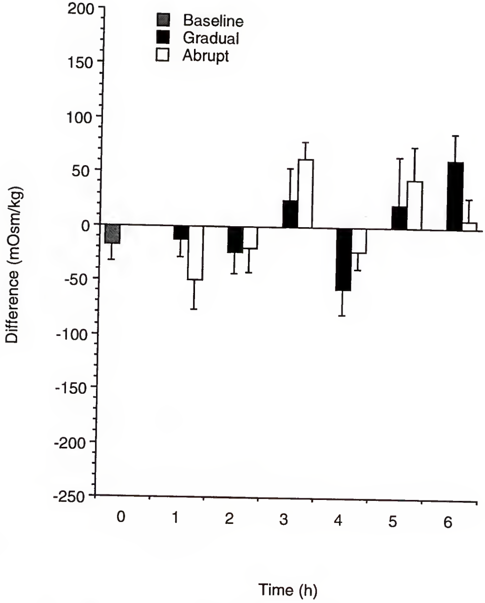

Figure 3-5. Difference in plasma osmolality from control values over six hours during salinity decreases.

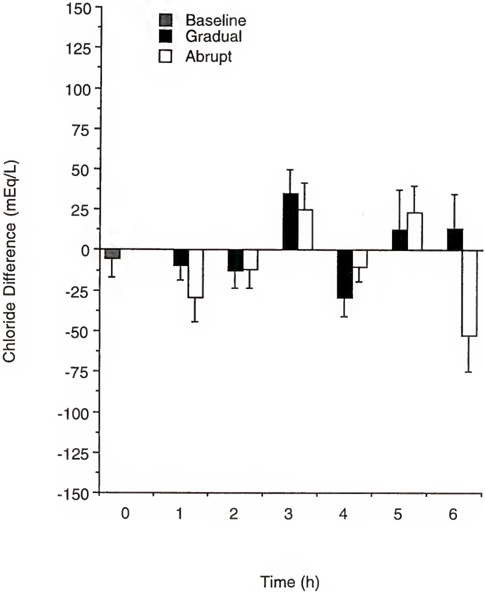

Figure 3-6. Difference in plasma chloride concentration from control values during salinity decreases.

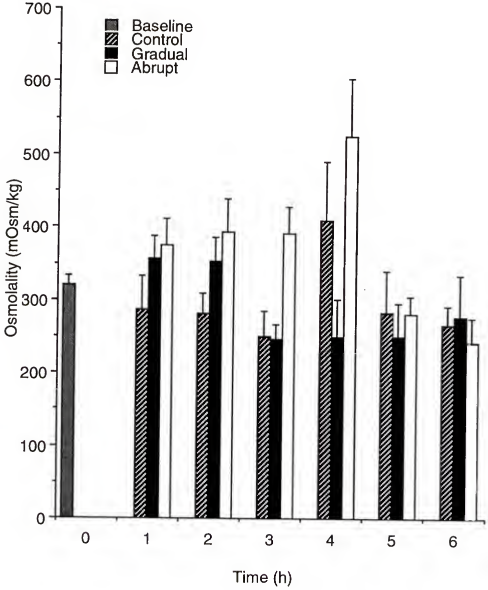

Figure 3-7. Plasma osmolality over six hours during salinity increases.

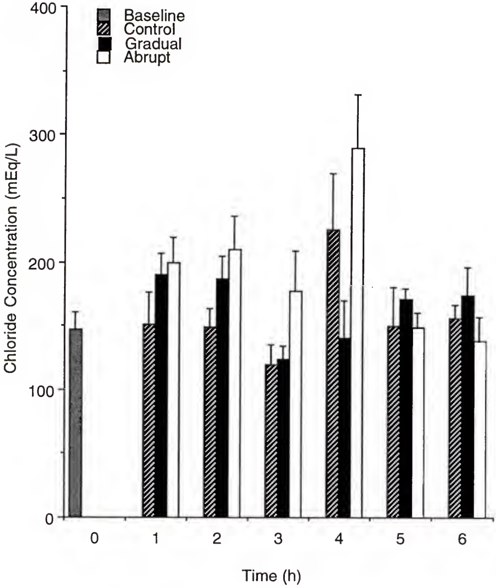

Figure 3-8. Plasma chloride concentration over six hours during salinity increases.

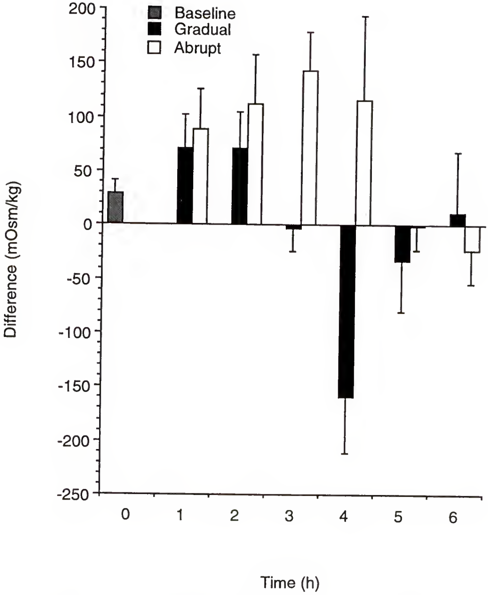

Figure 3-9. Difference in plasma osmolality from control values over six hours during salinity increases.

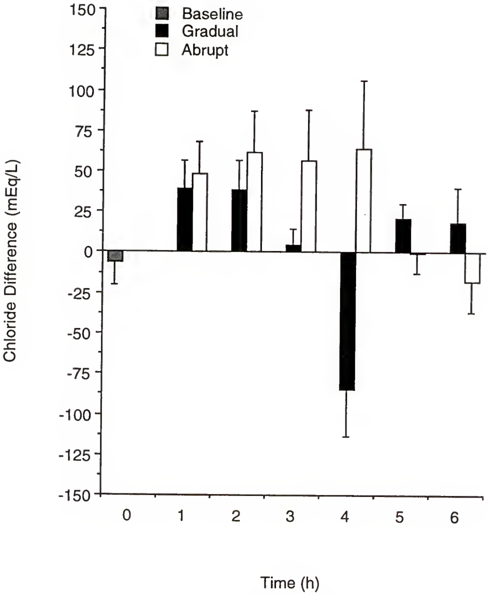

Figure 3-10. Difference in plasma chloride concentration from contol value over six hours during salinity increases.

Discussion

Although the slope of the regression line relating plasma osmolality to environmental osmolality for field-caught animals is not different from zero and indicates that these animals are osmoregulating across a wide range of environmental conditions, the osmolalities of different individuals at the same environmental value are highly variable. In comparison to previous blood chemistry measurements made by Nordlie and Leffler (1975) on juvenile mullet (10 to 37 g in mass), these results indicate that animals in the field regulate their plasma osmolality at higher values than do fish held in the laboratory under stable conditions. Within the range of the salinities used in both studies (100 to 1000 mOsm/kg), the plasma osmolality of field animals was 319 ± 7.6 mOsm/kg (Mean ± 1 SEM); the plasma osmolality of laboratory animals under stable conditions was 261 ± 4.4 mOsm/kg. The overall plasma osmolality of the fish used in the blood chemistry experiments presented here was 311 ± 5.8 mOsm/kg; however, removal of the baseline and control animals from this overall distribution resulted in a distribution of 305 ± 11.5 mOsm/kg. These findings suggest that laboratory manipulations like the ones used in this study are likely to yield results that better reflect the situation experienced by fish in their natural habitat.

The range of fish caught in this phase of the study only spanned about a two fold difference in size; yet, a significant size-related pattern was observed. An additional regression analysis conducted on mean plasma osmolality after animals were categorized into 2 cm size classes also produced a similar negative relationship between size and plasma osmolality. It is likely, however, that this relationship is largely determined by the outlying values in the smallest (8 to 10 cm) and largest (16 to 18 cm) size classes, and both of these size classes contain fewer animals than the intermediate size classes. Whether smaller fishes are simply more tolerant of higher plasma osmolalitites or are incapable of regulating as well as larger animals is unclear. Nordlie et al. (1982) found that smaller striped mullet (2.0 to 2.9 cm TL) were incapable of tolerating fresh water, whereas larger animals (3.0 to 6.9 cm) were able to successfully regulate at this low salinity.

Overall, the regression analyses suggest that the osmoregulatory abilities of these fish are variable and may be influenced by a number of factors. Because the environmental history of the field-caught animals is unknown, it is difficult to determine the relative roles of environmental influences and physiological abilities in the observed patterns.

In general, the data obtained from the fish in the decrease-change portions of these experiments were more variable than those obtained during the increase-change portion. The matched-subject protocol used in all of the experiments made it unlikely that factors were systematically and individually affecting the treatment and control groups, and the results of all ANOVA procedures using length as the dependent variable demonstrated that the matched-subject design was effective.

The data are interpreted to indicate that, during increasing salinity conditions, the abrupt increase produced a more intense and more persistent effect, relative to the gradual increase, that seems to be compensated for by the fifth hour. The gradual-change group data for both measures suggest over-compensation by these animals to this treatment at about four hours that is moderated in hours five and six. The patterns of total-osmotic and chloride concentrations shown by both the abrupt-change and gradual-change groups during salinity increases suggest that these types of salinity changes may pose a formidable challenges to the animal's regulatory mechanisms. In contrast, neither abrupt-change nor gradual-change salinity decreases seemed to perturb blood chemistry to the same extent. It may be easier for these animals to step mechanisms down to match the decreasing gradient than for them to activate the same, or altogether different mechanisms to work against an increasing gradient.

CHAPTER 4
EFFECTS OF ABRUPT AND GRADUAL SALINITY CHANGES ON THE METABOLISM OF JUVENILE STRIPED MULLET

The regulation of the internal body-fluid concentration near some stable value in the face of varying external medium concentration by fishes requires that these animals make adjustments to the rates of physiological processes. The work equation, presented in Chapter 1, indicates that these adjustments can be grouped into two categories: 1) the animal may vary the characteristics of its exchange surfaces to compensate for changes in the gradient or 2) the animal may alter the amount of work done in opposition to the changing diffusion rate brought about by the changing magnitude of the gradient. A reflection of this change in work would be a change in the overall metabolic rate of the animal. This chapter presents two empirical studies that were designed to estimate the magnitude of metabolic changes that are induced by short-term changes in salinity. The first study examined the influence of these salinity changes on the aerobic component of metabolism; the second study investigated the role of anaerobic processes in responding to these changes.

Methods

Over 100 juvenile mullet (Mugil cephalus) were collected using the same methods and from the same sites as the fish used in the blood chemistry experiments described in Chapter 3. The total lengths of these animals were all within the range of the larger sample used in previous experiments. Twenty-five fish were transported back to the aquarium room in the Zoology Department at the University of Florida where they were held for 24 hours in water obtained at the collection site. These animals were divided into two groups by matching according to total length. One group was held in well-aerated and filtered water with a total osmolality of approximately 1000 mOsm/kg for a minimum of one week in a 20 L glass aquarium (high group). The other group was held in well-aerated and filtered water with a total osmolality of about 100 mOsm/kg for the same duration in a 20 L glass aquarium (low group). Both groups were held at approximately 25 ° C on a 12 h light: 12 h dark cycle that began at 0800. These animals were subsequently used in the oxygen consumption experiments that are described below.

The remaining 65 fish were transported to the Whitney Laboratory for use in the lactate concentration experiments that are also described below. These animals were divided into two groups (high and low) by matching according to total length, and were held for a minimum of one week in water with a total osmolality of approximately 1000 mOsm/kg and 100 mOsm/kg respectively. Animals were held in these conditions in well-aerated water for, at least, a one week period before subsequent experimentation was conducted. All experiments were conducted at approximately 25 °C between 1000 h and 1900 h during the light phase a 12:12, light:dark cycle that began at approximately 0700 h.

Oxygen Consumption Measurement Protocol

At the end of the one week holding period, 10 animals from the high group and 10 animals from the low group were selected for use in subsequent oxygen consumption experiments. The remaining animals were held in a separate aquarium until these experiments were completed. In order to habituate the experimental animals to the measurement apparatus, each animal was transferred to another aquarium with water of the same osmotic concentration as its holding tank and placed into a section of grey opaque PVC pipe that was approximately 15 cm in diameter and approximately 25 cm long. The pipe was screened at both ends to prevent the animal's escape but allow aquarium water to flow through. The animal remained in the PVC pipe for 15 minutes before being returned to its holding aquarium. Each of the 20 animals, 10 from the high group and 10 from the low group, was transferred for 10 successive days. On the eleventh day, both ends of the PVC pipe were sealed with a plastic barrier connected to flexible plastic tubing that allowed water to be forced through the pipe-section at a rate that could be controlled by a variable-speed peristaltic pump. The animal remained in the experimental chamber, which was immersed in a 20L aquarium to provide thermal stability, for 30 minutes while well-aerated water with the same osmotic concentration as its holding tank was permitted to flow through. This procedure was repeated for five successive days. On the sixth day, the animal remained in the chamber for one hour. At the end of this interval, separate water samples were drawn into luer-tip syringes from the in-flow and out-flow sides of the flow-through apparatus. These samples were analyzed for oxygen content using the procedure described below, and difference in these two values was used to calculate the baseline rate of oxygen consumption under these stable salinity conditions.

For three additional days, each animal remained in the sealed flow-through experimental chamber for six hours per day. Each animal was subjected to a randomized order of the three treatments (control, abrupt-change, and gradual-change) over these days. On the control day, the water flowing through the chamber remained at the same osmotic concentration over the six hour interval. On the abrupt-change day, the osmotic concentration of the water flowing through the chamber was switched at the start of the experiment, either low-to high or high-to-low, depending on the concentration of the animal's holding tank. On the gradual-change day, the water at the start of the experiment was the same osmotic concentration as that in the holding tank, but the concentration was altered over the ensuing six hours so that, by the interval's end, the water had reached the other osmotic extreme. Water samples were drawn from both the in-flow and out-flow sides of the apparatus for oxygen content analyses at each of the one-hour intervals. At the end of each experiment, the animal was returned to its original holding tank.

All water samples were analyzed for oxygen content by injection into the sample chamber of an oxygen electrode (Model E5046, Radiometer Instruments, Copenhagen); the results were displayed on a acid-base analyzer (Model PHM-71 with PHA 930 PO_2 module, Radiometer Instruments, Copenhagen). The oxygen electrode was calibrated between 0 mm Hg (using a 0.1 Borax solution saturated with sodium sulfite) and full saturation (accounting for barometric pressure, relative humidity, temperature, and salinity) at the start and end of each experiment. All oxygen consumption measurements were corrected against values obtained from the experimental set-up without a fish in the chamber, but with a corresponding salinity regime. The actual chamber volume for each animal was determined by filling the chamber with water while the animal was present, removing the animal, and pouring the remaining fluid into a graduated cylinder so that the data could be adjusted for differences due to the animal's volume.

Lactate Concentration Measurement Protocol

Ten animals from each holding group (high or low) were assigned to one of three conditions (control, gradual-change or abrupt-change) by matching animals according to their total lengths. Each group was transferred to a well-aerated opaque-white plastic container (approximately 20 L) at the start of the experiment. Ten control animals were placed into a container with water that had the same osmotic properties as the water in which they were held during the preceding holding period, and this water remained the same for the next six hours. Abrupt-change animals were placed into a container with water of the other osmotic extreme (low-to-high or high-to-low transfer) at the start of the experiment, and this water remained the same for the next six hours. Gradual-change animals were placed into a container that had the same osmotic properties as the holding condition, but this water was replaced at a constant flow-rate over the next six hours so that, by interval's end, these animals were immersed in water of the other osmotic extreme.

At hours one and two from the start of the experiment, five of the animals from each group were removed from the experimental chamber. Each animal was decapitated, and the gill arches of both the right and left branchial chambers were dissected out and immediately stored in liquid nitrogen. A section of skeletal muscle, including both hypaxial and epaxial tissue, was also dissected and stored separately in liquid nitrogen. Both the gill and muscle tissue samples were returned to the laboratory in Gainesville and stored at -80 °C until processed for lactate concentration.

In order to determine lactate concentrations, gill filaments were separated from the gill arches and muscle tissue was separated from any associated bone. These frozen gill and muscle tissue samples were weighed and then transferred to a test tube where they were ground in 10 percent trichloroacetic acid (TCA) for 1 minute using an electric tissue grinder (Biospec Products, Bartlesville, Ok.). The resulting liquid was deproteinized by dilution with an equal volume of 10 percent trichloroacetic acid. Any remaining tissue was weighed, and its mass was subtracted from the initial sample mass to get a corrected sample mass. Lactate concentrations were determined using a commercial kit procedure (Procedure 826-UV, Sigma Chemical Co.); the assay depends on the ability of lactate dehydrogenase to use lactate present in the sample as a substrate for conversion of nicotinamide adenine dinucleotide (NAD) to its reduced form. Sample lactate concentrations were obtained from comparison to a standard curve generated with known lactate concentrations at the reaction's start and final UV (340 nM) absorption readings which correspond to end-point NADH concentrations.

Results

The number of observations in each cell of the three-factor (Change x Group x Time) mixed design used to analyze the effects of salinity change on oxygen consumption, with Change serving as a between-subjects factor and Group and Time serving as within-subjects factors, is given in Table 4-1. Results of the three-factor ANOVA are presented in Table 4-2. No significant Change x Group x Time interaction was found, but all three of the two-factor interaction terms were found to be significant. Post-hoc comparisons using the Scheffe test (Keppel, 1973) were used to identify significant differences between interaction groups. Data for the two change conditions (decrease and increase) are presented separately in Figures 4-1 and 4-2, respectively. The data for each experimental group (abrupt-change or gradual-change) at each

Table 4-1. Three-factor mixed design ANOVA used to determine the pattern of change in oxygen consumption in juvenile striped mullet (Mugil cephalus) subjected to either abrupt or gradual changes in salinity. Each cell gives the number of observations for a given fish for a given change x (subject x group x time) condition.

Decrease

	Abrupt	Control	Gradual
Time (hrs)	1 - 6	1 - 6	1 - 6
Subject			
1	6	6	6
2	6	6	6
3	6	6	6
4	6	6	6
5	6	6	6
6	6	6	6
7	6	6	6
8	6	6	6
9	6	6	6
10	6	6	6

Increase

	Abrupt	Control	Gradual
Time (hrs)	1 - 6	1 - 6	1 - 6
Subject			
1	6	6	6
2	6	6	6
3	6	6	6
4	6	6	6
5	6	6	6
6	6	6	6
7	6	6	6
8	6	6	6
9	6	6	6
10	6	6	6

Table 4-2. Results of three-factor mixed design ANOVA, Change x (Group x Time), for oxygen consumption of juvenile striped mullet (Mugil cephalus).

Source	df	F	P
Change	1	4.53	<0.050
Error-Between	18		
Group	2	3.36	<0.050
Time	5	4.12	<0.010
Change x Group	2	3.26	<0.050
Change x Time	5	2.98	<0.025
Group x Time	10	2.27	<0.025
Change x Group x Time	10	0.64	NS
Error - Within	306		

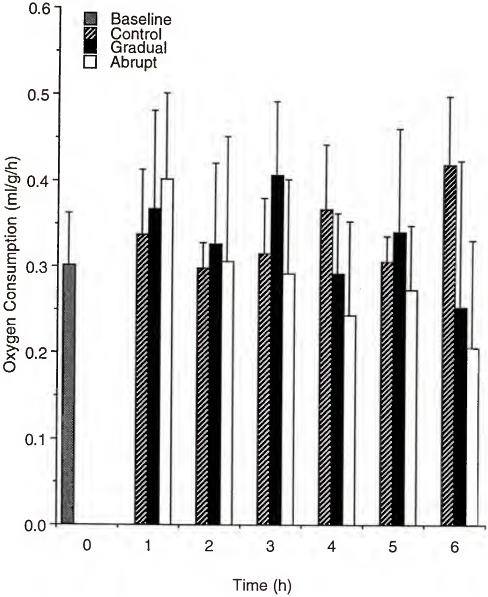

Figure 4-1. Oxygen consumption over six hour during salinity decreases

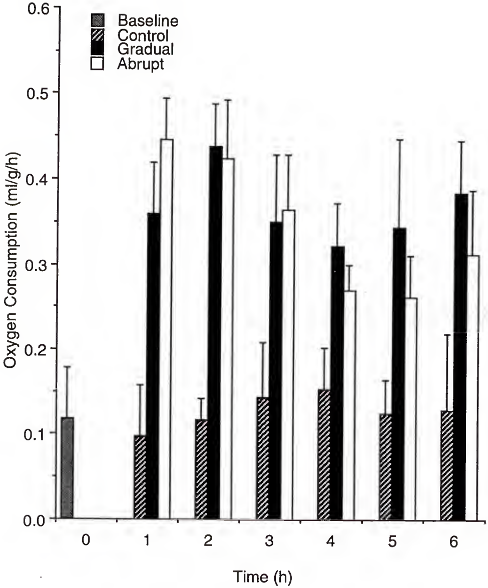

Figure 4-2. Oxygen consumption over six hour during salinity increases

time-interval (hours 1 through 6) are referenced to their respective control group and presented separately for each change condition in Figures 4-3 and 4-4. For the decrease-change group, no differences between the control and experimental groups were detected, and the control groups at each time interval seem to be as different from one another as they are from any of the experimental groups. In contrast, the oxygen consumption values of the experimental groups in the increase-change condition show a significant departure from their respective control groups, particularly in the earlier intervals (hours 1 - 3). No significant differences, however, were found between the abrupt-change and gradual-change groups within a single time interval.

The number of observations in each cell of the four-factor (Change x Group x Time x Tissue) mixed design used to analyze the effects of salinity change on lactate concentration, with change, group and time serving as between-subjects factors and tissue serving as a within-subject factor, is given in Table 4-3. Results of the corresponding four-factor ANOVA indicated no significant effects for any of the main factors or interaction terms. In order to increase the statistical power of the analysis by increasing the sample size of each change x group x tissue cell, the data from each time interval (hours 1 and 2) were pooled. The resulting three-factor (Change x Group x Tissue) mixed design is presented in Table 4-4. Results of the corresponding ANOVA are presented in Table 4-5. No significant effects were found for any of the main factors or interaction terms. Group x Tissue cell means for the decrease-change and increase-change conditions are presented separately in Figures 4-5 and 4-6.

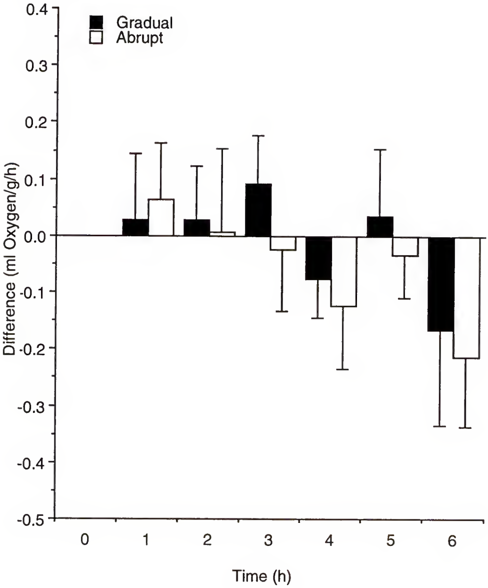

Figure 4-3. Difference in oxygen consumption from control value over six hours during salinity decreases.

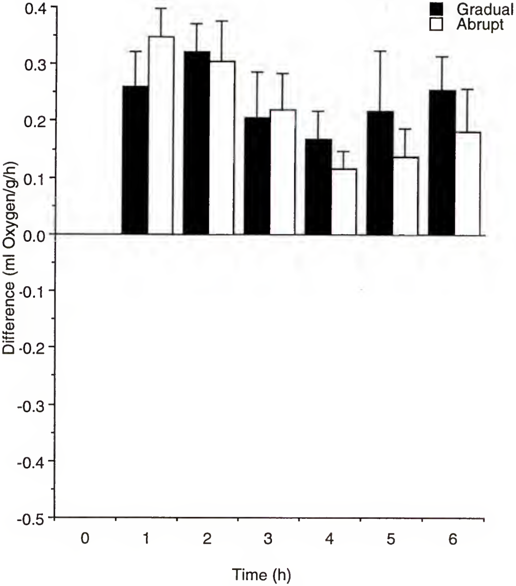

Figure 4-4. Difference in oxygen consumption from control value over six hours during salinity increases.

Table 4-3. Four-factor mixed design ANOVA used to determine the pattern of change in lactate concentration of gill (G) or muscle (M) tissue in juvenile striped mullet (Mugil cephalus) subjected to either abrupt or gradual changes in salinity. Each cell gives the number of observations for a given change x group x time(subject x tissue) condition.

	Decrease											
	Abrupt				Control				Gradual			
Time (hrs)	1		2		1		2		1		2	
Tissue	G	M	G	M	G	M	G	M	G	M	G	M
Subject												
1	1	1	1	1	1	1	1	1	1	1	1	1
2	1	1	1	1	1	1	1	1	1	1	1	1
3	1	1	1	1	1	1	1	1	1	1	1	1
4	1	1	1	1	1	1	1	1	1	1	1	1
5	1	1	1	1	1	1	1	1	1	1	1	1

	Increase											
	Abrupt				Control				Gradual			
Time (hrs)	1		2		1		2		1		2	
Tissue	G	M	G	M	G	M	G	M	G	M	G	M
Subject												
1	1	1	1	1	1	1	1	1	1	1	1	1
2	1	1	1	1	1	1	1	1	1	1	1	1
3	1	1	1	1	1	1	1	1	1	1	1	1
4	1	1	1	1	1	1	1	1	1	1	1	1
5	1	1	1	1	1	1	1	1	1	1	1	1

Table 4-4. Three-factor mixed design ANOVA used to determine the pattern of change in lactate concentration of gill (G) or muscle (M) tissue in juvenile striped mullet (Mugil cephalus) subjected to either abrupt or gradual changes in salinity. Each cell gives the number of observations for a given change x group x (subject x tissue) condition.

Decrease

	Abrupt		Control		Gradual	
Tissue	G	M	G	M	G	M
Subject						
1	1	1	1	1	1	1
2	1	1	1	1	1	1
3	1	1	1	1	1	1
4	1	1	1	1	1	1
5	1	1	1	1	1	1
6	1	1	1	1	1	1
7	1	1	1	1	1	1
8	1	1	1	1	1	1
9	1	1	1	1	1	1
10	1	1	1	1	1	1

Increase

	Abrupt		Control		Gradual	
Tissue	G	M	G	M	G	M
Subject						
1	1	1	1	1	1	1
2	1	1	1	1	1	1
3	1	1	1	1	1	1
4	1	1	1	1	1	1
5	1	1	1	1	1	1
6	1	1	1	1	1	1
7	1	1	1	1	1	1
8	1	1	1	1	1	1
9	1	1	1	1	1	1
10	1	1	1	1	1	1

Table 4-5. Results of three-factor mixed design ANOVA, Change x Group x (Tissue), for lactate concentration of juvenile striped mullet (Mugil cephalus).

Source	df	F	P
Change	1	3.904	NS
Group	2	0.080	NS
Change x Group	2	0.313	NS
Error-Between	54		
Tissue	1	3.167	NS
Change x Tissue	1	3.555	NS
Group x Tissue	2	0.629	NS
Change x Group x Tissue	2	0.271	NS
Error - Within	54		

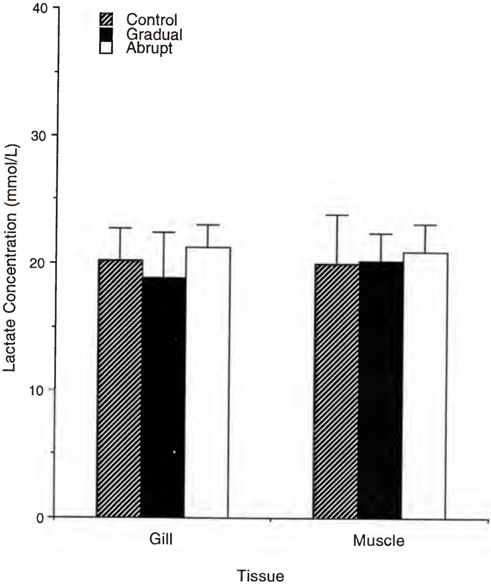

Figure 4-5. Average lactate concentration in gill and muscle tissue combined for hours one and two during salinity decreases.

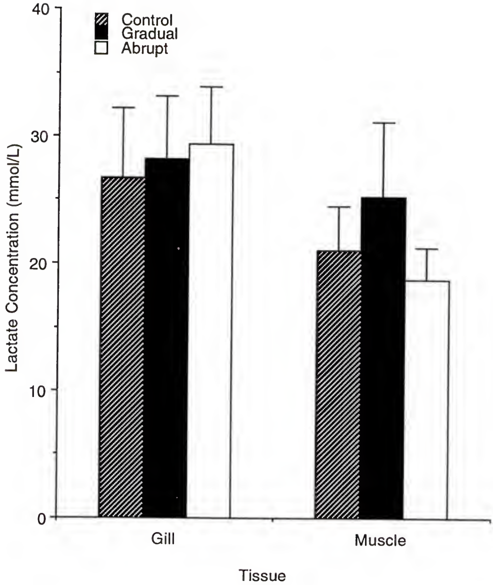

Figure 4-6. Average lactate concentration in gill and muscle tissue combined for hours one and two during salinity increases.

Discussion

The overall results of the oxygen consumption experiments would suggest that salinity effects are different depending on whether the animal is experiencing a salinity increase or decrease. Nordlie and Leffler (1975) measured the oxygen consumption rates of striped mullet (Mugil cephalus) under steady-state conditions across the full range of salinity used in this study. Figure 4-7 compares the results of the present study during salinity decreases with the corresponding measurements made under steady-state conditions. Although the animals under short-term salinity decreases began the experiment with oxygen consumption rates that were actually below those of animals held at the same salinity for two weeks by Nordlie and Leffler (1975), the animals in the control group and both experimental groups had oxygen consumption rates that were all above those of steady-state animals at corresponding salinities, and all three groups showed a great deal of nonsystematic variation in oxygen consumption rates over time.

Figure 4-8 compares the results obtained from animals during short-term salinity increases with those obtained from fish under steady-state conditions at corresponding salinities. Control animals seem to maintain oxygen consumption rates that are below those of steady-state animals across the entire six-hour interval. Both gradual and abrupt increases in salinity seem to induce a significant increase in aerobic metabolism over the first two hours; however, rates at hour three seem to be declining toward those obtained under steady-state. The pattern shown by both gradual-change and abrupt-change animals during hours four through six seems consistent with the pattern shown by steady-state animals at comparable salinities, suggesting that these animals

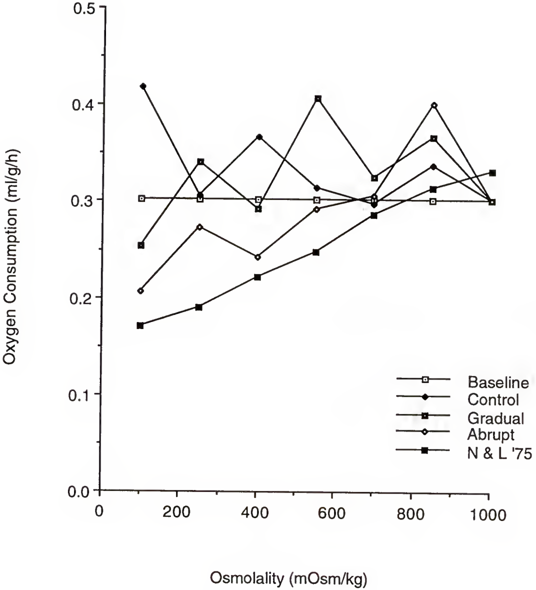

Figure 4-7. A comparison of oxygen consumption during abrupt and gradual salinity decreases with steady-state values obtained by Nordlie & Leffler (1975) at the same salinity.

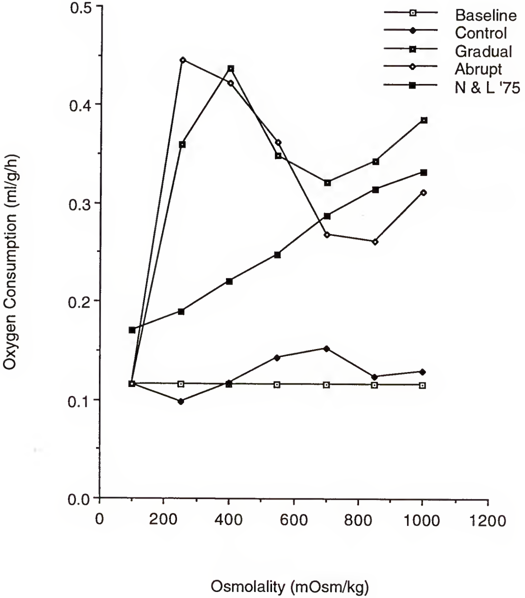

Figure 4-8 A comparison of oxygen consumption during abrupt and gradual salinity increases with steady-state values obtained by Nordlie & Leffler (1975) at the same salinity. The oxygen consumtion rates of the abrupt-change animals at salinities between 250 and 400 mOsm/kg and the gradual-change animals at 400 mOsm/kg are different than the oxygen consumption rates of control animals at comparable salinities.

may have fully overcome the metabolic challenge brought on by these salinity changes.

Although the lactate concentration values of the control and experimental groups do not differ from one another under either short-term decreases or short-term increases in salinity, the lactate concentration values obtained from this study are quite high in comparison to other species in which acidosis was induced by stimulating the animals to exhausting muscular activity (see Holeton and Heisler, 1983; Toews et al., 1983). Tissue samples were collected in this study at hours one and two because the exercise-based studies indicated that lactate wash-out might be maximal between two and five hours, and the results of the blood chemistry analyses (see Chapter 3) suggested that this species probably begins to compensate for changes in internal concentration shifts, brought about by changes in the external medium's concentration, even earlier.

The uniformly high lactate concentration values seen in these fish are difficult to interpret. Nordlie and Leffler (1975) have noted that striped mullet show high rates of aerobic metabolism in comparison to other fishes. It may be that these fish also show high rates of anaerobic metabolism. Alternatively, the handling procedures used in this study may be preferentially inducing increases in anaerobic metabolism, but not aerobic metabolism, that over-ride any salinity effects. Unfortunately, no tissue samples were taken from animals before they were assigned to the different groups, so at present, anaerobic metabolic changes that might occur earlier than one hour in this species are unknown.

CHAPTER 5
SUMMARY AND CONCLUSIONS

The patterns of salinity manipulations (maximum-tidal abrupt and gradual changes) chosen for this study are thought to represent two extremes that striped mullet (Mugil cephalus) may encounter in the estuarine habitats that they frequent as juveniles. Because of the large quantities of water needed to make these salinity manipulations, it was necessary to rely on the locally available well water. Except in cases where episodic events may flood estuarine habitats with rain-water that is hypo-osmotic to the normal ground water, this well water is probably very similar, in terms of its osmotic and ionic properties, to the minimum concentration values that these fish may regularly experience. Figure 5-1 depicts the change in the concentration gradient expected as the external medium's concentration fluctuates between that of open-ocean water and the ground water that was available (approximately 100 mOsm/kg). Figure 5-2 illustrates the effect on the work function of this change in the minimum tidal value from fresh water (5 mOsm/kg) to one-tenth seawater (100 mOsm/kg). The most apparent difference between the idealized case and the realized one is that a relatively small change in the minimum osmotic concentration can effect a very large alteration in the relatively expensive portion of the work function that corresponds to regulation in the lower concentration medium. This effect is functionally similar to the one that Potts proposed was operating in many freshwater and brackish water invertebrates;

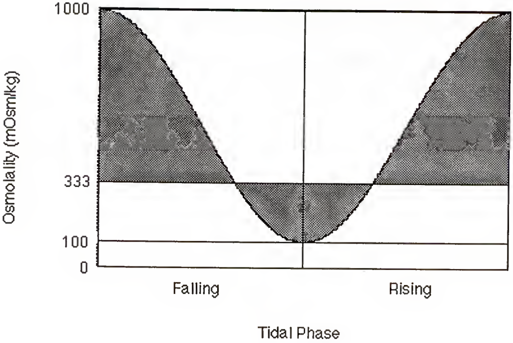

Figure 5-1. The osmotic concentration of fish blood plasma (333 mOsm/kg) relative to the maximum (1000 mOsm/kg) and minimum (100 mOsm/kg) external medium concentrations in the vicinity of the Whitney Laboratory during a maximum-amplitude tidal cycle. The shaded area illustrates the difference (gradient) between the animal's internal osmotic concentration and the osmotic concentration of the external environment.

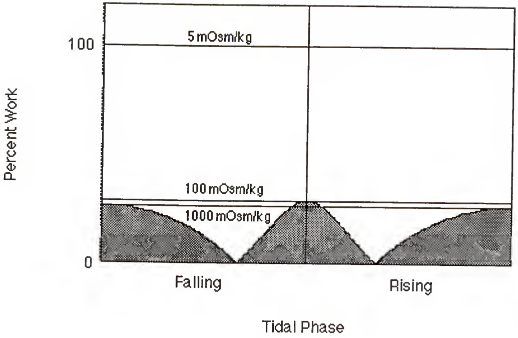

Figure 5-2. Work required for stable regulation of internal concentration at 333 mOsm/kg as a function of a sigmoidal change in the medium concentration between 1000 mOsm/kg and 100 mOsm/kg. Work is expressed as percent relative to the work required for stable regulation under freshwater conditions (5 mOsm/kg) as calculated from the work equation ($W = R \times T \times P \times | \ln C_{int} - \ln C_{ext} |$). The effect of a minimum concentration of 100 mOsm/kg is illustrated by a lower peak in the work function at the tidal minimum (between the falling and rising phases) when compared to the peak in Figure 1-2 (p. 10). The work value at the tidal maximum (1000 mOsm/kg) is nearly equivalent to the work value at the tidal minimum (100 mOsm/kg).

it reduces the magnitude of the gradient, and it suggests that a premium should be placed on the avoidance of very dilute media.

The results of the blood chemistry analyses conducted on samples obtained from fish in the field describe a distribution of internal osmotic concentrations that is both higher and more variable than the distribution obtained previously from striped mullet held in the laboratory at comparable but constant salinities for two weeks (Nordlie and Leffler, 1975). Figure 5-3 illustrates these two distributions (Mean ± 2 SEM) relative to the total range of external osmotic concentrations used for the experimental manipulations. These two distributions can be compared with the blood chemistry results obtained from the short-term salinity changes.

The results of the empirical study described in Chapter 3 suggest that juvenile striped mullet are susceptible to changes in their internal fluid concentration that are brought about by changes in the external medium concentration. The work equation provides a way to evaluate the predicted effect that these observed changes in internal concentration should have relative to an idealized situation where regulation remains very tight. Figure 5-4 depicts the difference in the mean osmotic concentration values obtained from the fish used in these experiments from the two expected distributions (field and stable-laboratory) under the abrupt square-wave function. During both the gradual-decrease (falling phase) and gradual-increase (rising phase) manipulations, the internal osmotic concentration of these animals changes in the direction of the imposed change in the external concentration early in the phase. In both phases, mean internal osmotic concentrations show a reversal in pattern towards the phase's end. These reversals probably reflect the actions of regulatory mechanisms in these animals.

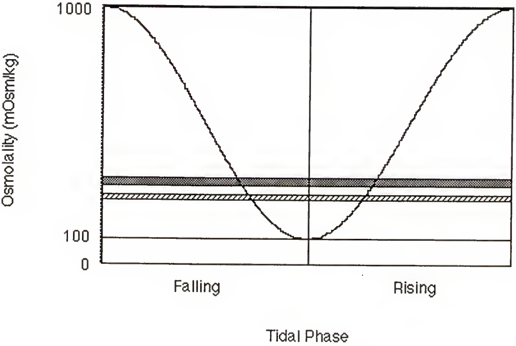

Figure 5-3. Plasma osmolality limits (Mean ± 2 SEM) for blood samples obtained from field animals (shaded band) and laboratory animals held at a constant salinity for two weeks prior to sampling (diagonal band; Nordlie and Leffler, 1975). The upper limit of the field animal distribution (top of shaded band) is equal to 334 mOsm/kg. The distribution of the field animals (319 ± 7.6) is broader than that of the laboratory animals (261 ± 4.4); however, both distributions are narrow in comparison to the range of external concentrations (1000 to 100 mOsm/kg) over which blood samples were obtained.

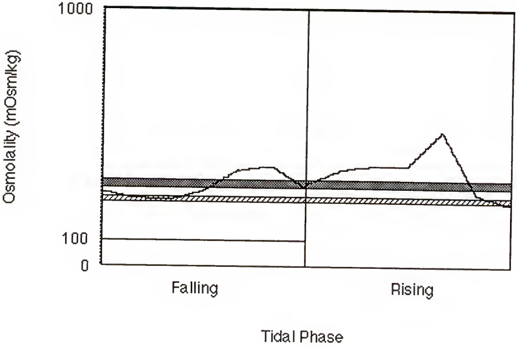

Figure 5-4. Comparison of mean plasma osmolality during the abrupt tidal cycle to plasma osmolality distributions (Mean ± 2 SEM) of field animals (shaded band) and stable laboratory animals (diagonal band; Nordlie and Leffler, 1975). External concentrations remained constant during each phase of the tidal cycle and are illustrated by the horizontal lines at 100 mOsm/kg (falling phase panel) and 1000 mOsm/kg (rising phase panel).

Figure 5-5 illustrates the pattern of expected work values calculated using the internal concentrations shown in Figure 5-4 in the work equation when the terms R, T, and P are held constant. Relative to the expected distribution in the work function calculated using the internal concentrations obtained from field animals (shaded band), the projected values suggest that the responses of the experimental animals should require less work early in the phase (both falling and rising) and more work towards the phase's end. These predictions do not agree with the results of the oxygen consumption experiments which showed that salinity increases (corresponding to the rising phase in Figure 5-5) were the only manipulations that produced significant increases in metabolism. The variability in the experimental data that extends beyond the limits of the ditribution for the field animals may be interpreted as indicating that these fish are making either physiological or behavioral adjustments under natural conditions which are not possible under the experimental conditions. One possibility is that field animals are selecting microhabitats with osmotic concentrations that help these animals maintain their internal concentrations within these narrower limits. Alternatively, the discrepancies in the predicted work function , relative to the oxygen consumption data, may be accounted for by a change in the permeability term in the work equation, presumed to reflect a change in the functional gill surface area (see Nordlie and Leffler, 1975).

The change in mean internal osmotic concentration of the experimental animals during the gradual-change manipulations are illustrated in Figure 6-6. The patterns observed during both the falling phase and rising phase of this cycle are similar to those obtained during the abrupt-change cycle, but the magnitude of the internal concentration increase during the rising phase of the abrupt change cycle (see right panel of Figure 5-4) seems to be larger and

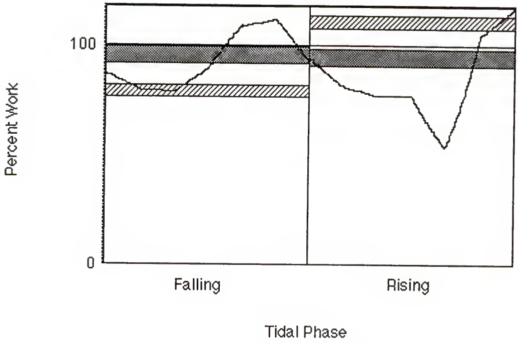

Figure 5-5. Comparison of predicted values for the abrupt tidal cycle with projected work distributions for field animals (shaded band) and stable laboratory animals (diagonal band; Nordlie and Leffler, 1975). Work was calculated form based on measured internal and external concentrations (shown in Figure 5.4) using the work equation ($W = R \times T \times P \times | \ln C_{int} - C_{ext} |$). Work is plotted relative to the work required for stable regulation at 334 mOsm/kg (the upper limit of the field distribution when regulating against an external concentration of 100 mOsm/kg). The figure illustrates that projected work is lower for stable laboratory animals at hypo-osmotic external concentrations and is lower for field animals at hyper-osmotic external concentrations.

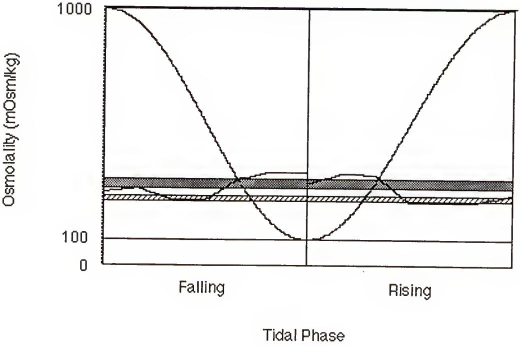

Figure 5-6. Comparison of plasma osmolality during the gradual tidal cycle with plasma osmolality distributions (Mean ± 2 SEM) of field animals (shaded band) and stable laboratory animals (diagonal band; Nordlie and Leffler, 1975). The external medium concentration is depicted by the sigmoidal line which ranges between 1000 and 100 mOsm/kg. Mean plasma osmolality for animals from the present study ranges between the two dustributions at hyper-osmotic external concentrations and exceeds both distributions at hypo-osmotic external concentrations.

to last longer than that observed during the rising phase of the gradual-change cycle.

The corresponding values in the predicted work function for the gradual-change osmotic concentration data are presented in Figure 5-7. The predicted net effect of the mean internal concentration values seen in the falling phase of this cycle is an increase in work relative to that expected from the field distribution (shaded band); the magnitude of this increase is predicted to be greater during that part of the interval when the animal's internal concentration is still below that of the external medium. Though the observed mean internal concentration during the early part of the rising phase is within the range of the expected work function for the field animals, the subsequent portion of the rising phase is dominated by an increase in the expected work function above that predicted for field conditions Whereas the projected work values would suggest that similar increases in metabolism might be observed during both the falling and rising phases of the gradual-change manipulations, the results of the oxygen consumption experiments indicate that only the increasing manipulation (corresponding to the rising phase in Figure 5-7) produces such metabolic changes.

According to the work equation

$$W = R \times T \times P \times | \ln C_{int} - \ln C_{ext} |$$

work (W) can be held constant during decreases in the magnitude of the gradient ($| \ln C_{int} - \ln C_{ext} |$) by increasing permeability (P). This increase in permeability (e.g., by increasing the surface area) would seem to be physiologically acceptable, as well, because the risk associated with the diffusional load is also decreasing. The increase in work associated with an

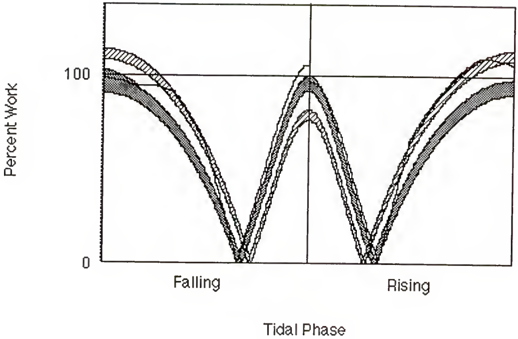

Figure 5-7. Comparison of projected work values for the gradual tidal cycle with projected work distributions for field animals (shaded band) and stable laboratory animals (diagonal band; Nordlie and Leffler, 1975). Work was calculated based on measured internal and external concentrations (shown in Figure 5-6) using the work equation ($W = R \times T \times P \times | \ln C_{int} - \ln C_{ext} |$). Work is plotted relative to the work required for stable regulation at 334 mOsm/kg (the upper limit of the field animal distribution when regulating against an external concentration of 100 mOsm/kg). The figure illustrates that the projected work distribution is lower for field animals at hyper-osmotic external concentrations and lower for stable laboratory animals at hypo-osmotic external concentrations. Projected mean work for the experimental animals from the present study ranges between the two distributions at hyper-osmotic external concentrations and exceeds both distributions at most hypo-osmotic external concentrations.

increase in the gradient potentially puts the organism at risk. If permeability is also increasing along with the gradient and work increases, the animal is subject to an even larger diffusional loss. On the other hand, if if the area term remains stable or decreases as both the gradient and work terms increase, the implication is that the animal is somehow supporting an increase in aerobic metabolism with a proportionally smaller surface area. Such an adjustment seems counter-intuitive when one considers that the most likely surface area being affected is that of the gills, which would also be involved in the gas exchange supporting the increase in aerobic metabolism. A more likely explanation for the increase in metabolism might be an increase in the activity of aerobically fueled transport-protein activity that is working against the diffusional load brought about by the gradient increase. The value in applying a model such as the thermodynamic work model to these sorts of physiological problems lies not so much in the model's ability to provide an accurate description of the system's behavior; rather, the model provides an organizational frame-work with which to restructure hypotheses about the system when the original formulation seems to be inadequate.

A comparison of the results obtained from this study with the results obtained from field animals or the more traditional experimental approach (i.e., one in which animals are permitted long acclimation periods to the different salinity conditions) suggests that each approach can be advantageous or disadvantageous depending on the information one is trying to obtain. The more traditional experimental approach seems to provide the best estimates of both the minimum internal osmotic concentration at which these animals are capable of regulating and the minimum energy expenditure required for this regulation when the animal is permitted to make all of the adjustments that are within its capability. However, this approach provides no information regarding the dynamic nature of the regulatory process.

Field data, at least those resulting from single time-point sampling, are difficult to interpret because they include no information about the animal's previous environmental history. They may, however, tell us about these animals' preferences when considered with respect to the different habitats these animals can access.

The experimental approach used in this study allows one to detect and describe the dynamic nature of the osmoregulatory process by removing the effects of large scale behavioural processes that may affect these measures (e.g., movement of animals between the different salinity zones). It also allows one to structure the animal's salinity history and assess the differential effects of these temporal patterns. The design seems to provide a better estimate of the mean level of regulation under field conditions relative to the design that employs longer acclimation times, but it also seems to yield results that are even more variable than are characteristic of field animals. The greatest utility of this approach, however, is that it gives one a means to begin to assess the effects of living in habitats that are themselves dynamic. The results of this study suggest that the temporal patterning of the osmotic environment may be an important component of these animals natural history. They provide preliminary evidence that habitat assessments should incorporate indices that take this temporal aspect of the environment into account.

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BIOGRAPHICAL SKETCH

Evan Chipouras was born Stylianos Papathanassiou around 29 November 1955 in Piraeus, Greece. He remained in the Athens Children's Asylum in Piraeus until the spring of 1957 when he first came to the United States, having been adopted by a Greek-American family, Peter and Ruth Chipouras. On 20 August 1959, Evan was granted U.S. citizenship. Evan graduated from an Atlanta, Ga. secondary school in June 1973, and he was admitted to the University of Georgia in Athens the following fall to pursue his bachelor's degree. Evan majored in psychology and received his Bachelor of Science degree in June 1977. Evan re-entered the University of Georgia a few years later and received his Master of Science degree in psychology in March 1985. His master's thesis compared the effects of sensory stimulation on the motor activity of young and old squirrel monkeys, Siamiri sciureus. While working on both his bachelor's and master's degrees, Evan took extensive course work in UGA's biology and zoology departments, and he developed a keen interest in the ecological physiology of fishes. With encouragement from his mentors; Walt Isaac, Professor of Psychology, and Joe Crim, Professor of Zoology, Evan decided to pursue this interest as the focus of his doctoral work. In August 1987, Evan was admitted to the University of Florida where he began working in the laboratory of David H. Evans. Evan conducted his doctoral research work both in the Zoology Department at UF and at the C. V. Whitney Marine Laboratory in St. Augustine, Fla. After receiving his Doctor of Philosophy degree from UF, he plans to continue his investigation into the role of environmental factors on the physiology of young estuarine-dependent fishes.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

David H. Evans, Chairman
Professor of Zoology

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Frank G. Nordlie

Frank G. Nordlie
Professor of Zoology

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William E. S. Carr

William E. S. Carr
Professor of Zoology

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Michele G. Wheatly

Michele G. Wheatly
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Daniel E. Canfield Jr.

Daniel E. Canfield Jr.
Professor of Fisheries
and Aquatic Sciences

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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This dissertation was submitted to the Graduate Faculty of the Department of Zoology in the College of Liberal Arts and Sciences and to the Graduate School and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

April, 1994

Dean, Graduate School